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SOIL TEMPERATURE VERSUS DROUGHT AS A FACTOR DETERMINING LOWER ALTITUDINAL LIMITS OF TREES IN THE ROCKY MOUNTAINS

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Introduction

Each of the various species of coniferous trees which dominate the Rocky Mountain forests has rather definite limits of altitude above and below which it normally does not grow, and these vary considerably among species. It seems safe to assume that the factors which govern the upper altitudinal limits of a tree are in all probability different from those which effect the lower limits of its range. For example, if the summer temperature above a certain elevation is insufficient to meet the heat requirements of a particular species, this factor cannot be effective in the same way at the lower limit of the species range, since temperature increases down the slope. It is therefore feasible to ignore one set of factors, such as those involved in determining the upper altitudinal limits, and concentrate attention on the other set. This paper is concerned primarily with the two factors most frequently suggested as playing a critical role in determining the downward limits of distribution, namely, high temperature and drought.

The earliest important contributor to the geography of the montane vegetation of the Rocky Mountain region was C. H. MERRIAM, who considered that the lower altitudinal limits of plants are determined by their varying abilities to endure midsummer heat. This critical degree of heat he contended could be expressed as the mean temperature of the six hottest consecutive weeks in summer. His assumption was wholly unsupported by experimental or circumstantial evidence, and although it was long accepted without question, the hypothesis (**7**) is now generally discredited.

Later investigators made sufficient measurements of the environmental factors associated with the zonal sequence of vegetation to hazard a guess as to which factor is most likely limiting in effect. Also, the interpretation of species behavior at the lower limits of altitudinal distribution proved an additional source of information. It is significant that practically all these contributors (**5, 10, 12, 16, 22, 23, 25, 26, 27,** etc.) concluded that drought is more important than high temperature in determining lower altitudinal limits. BATES (**1**), however, considered lack of shade for the seedlings the factor most limiting in the reproduction of Douglas fir (*Pseudotsuga taxifolia*) at its lower altitudinal limits.

Experimentation has been employed in connection with Rocky Mountain vegetation zones only within the last two decades. To date two investigators, BATES and PEARSON, have made the principal experimental contributions to the etiology of this zonal system, but their results have not led to the same conclusions with respect to lower altitudinal limits. PEARSON held that the lower limits of distribution are determined by the varying abilities of species to withstand drought, while BATES placed more importance on high temper-

ature at the soil surface. The present experimental study attempts to determine the relative importance of these two factors.

By exposing flats of seedlings to strong insolation, BATES (2) observed differences in mortality which indicated that seedlings of Engelmann spruce (*Picea engelmannii*) and lodgepole pine (*Pinus murrayana*) are more susceptible to high soil surface temperatures than are Douglas fir and ponderosa pine (*Pinus ponderosa*). This finding seems to have significance in connection with distribution, in view of the fact that the latter two species range down to distinctly lower elevations in the Rockies than do the former.

Later, by using a different technique, somewhat different results were obtained (4). In these experiments potted seedlings were exposed successively when they were 46, 64, 90, and 92 days old to heat radiated from a resistance coil mounted under a reflector. To make sure that soil drying was not a cause for mortality, the soil in the pots was watered so thoroughly that its surface temperature never became as hot during the subsequent tests as did the adjacent layer of air, and in all cases foliage injury rather than stem injury at the ground level was observed. This second study, therefore, resolved itself to tests of the relative abilities of different species of seedlings to tolerate the effects of hot air about their foliage. According to the results obtained, the species could be rated as to sensitiveness as follows (the figures which follow the names indicate the order in which the species are normally encountered in ascending a slope):

Most sensitive:	Lodgepole pine.....	3
	Ponderosa pine.....	1
	Englemann spruce....	4
Least sensitive:	Douglas fir.....	2

Since in this experiment the relative tolerance of the foliage of the seedlings to high temperature bears no relation to their zonal distribution, it would appear that this aspect of high temperature is not critical in determining the lower altitudinal limits of the species. However, 2 years later BATES (3) still maintained that the forest zones of the central Rockies "are essentially temperature belts and only secondarily moisture belts," and explained the difference in the lower altitudinal limits of Douglas fir and ponderosa pine as due to an ability of seedlings of the latter species to tolerate higher levels of temperature at the soil surface. The temperature at this level, he stated, is "far more important" than air temperatures

A contrasted view is held by PEARSON (19). He transplanted small trees of Douglas fir and blue spruce (*Picea pungens*), which have approximately the same altitudinal distribution, into the ponderosa pine zone. All transplants succumbed in such a manner as to lead PEARSON to conclude that drought was the causal factor, and he explains the differing drought tolerance among species as due to the relative depth of the root systems in relation to the periodic desiccation of the upper soil horizons. Moreover, he pointed out that all forest trees native to the southern Rockies can grow at altitudes much lower than they naturally occur if they are watered adequately.

It was the purpose of the research reported in the present paper to make comparative studies under controlled conditions of both heat and drought tolerance of certain of the more important coniferous tree species of the Rockies, and then, where advisable, to compare these results with measurements of the same factors under field conditions. The former

type of study should provide a quantitative physiological basis for evaluating the relative importance of the accumulated field data and thus contribute information which may help to clear up the existing uncertainty concerning this phase of forest distribution.

Investigation

Relative tolerance of high soil-surface temperatures

Methods.—The species used in this study, together with the seed source, in the order of their zonal relationships, are as follows:

Subalpine fir (*Abies lasiocarpa*)—San Isabel National Forest, Colorado
Engelmann spruce—San Isabel National Forest, Colorado
Douglas fir—McCall, Idaho[1]
Ponderosa pine—McCall, Idaho
Piñon (*Pinus edulis*)—Colorado

The seeds were planted in a heavy sandy loam surfaced with 1 cm. of sand. At the time of planting, cupric oxalate was applied to the soil surface at the rate of 5 gm. per square foot to minimize losses due to damping-off fungi, and the soil was soaked with a complete nutrient solution at that time.

The flat measured 20 × 120 cm., and the seeds were sown in mixed rows approximately 2 cm. apart down the length of the flat. Just before the tests were begun all misformed and under-developed seedlings were removed, and the remaining plants were thinned so that no part of the soil surface would be especially shaded by the foliage of a clump of seedlings.

The duration of the heat treatments was arbitrary, although an attempt was made to simulate field conditions. Seedlings which germinate in forest openings are subjected each day to periods of insolation, the lengths of which depend upon the size and position of nearby objects which can intercept direct sunlight. During the day the temperature of the soil surface rises and falls with each moving shadow and passing cloud. To approximate these conditions in the greenhouse, each temperature test was conducted for a 6-hour period in the middle of the day, and at an average of once every 5 minutes the heating apparatus was connected until the surface temperatures rose to the desired level, at which time it was disconnected to prevent overheating. Thermostatic control of heat may have had some advantages in this connection, but sustained high temperature levels would be very unlike field conditions; moreover, thermoregulators to control true surface temperatures were unavailable. The temperature—even a few millimeters above or below the soil surface—is very different from that of the surface layer of soil particles, and it is the temperature of this surface which is critical. Temperatures were measured with a copper-constantan thermocouple,[2] with the measuring junction barely covered by the surface layer of sand grains. The accuracy of this instrument was checked by use of hot-water bath immediately before each test. Throughout each 6-hour period the writer personally tended the apparatus, bringing the temperature up to the desired level every few minutes.

Heat treatments were begun when the seedlings were 47 days old. The tests were made on alternate days, each time elevating the temperature level by 5° C.,

[1] Acknowledgment is hereby made of the generous co-operation of Mr. Royale K. Pierson, Mr. T. B. Glazebrook, and Mr. J. E. King of the School of Forestry, University of Idaho, for supplying and stratifying certain of the seeds used in this study.

[2] The writer is greatly indebted to Dr. Hobart Beresford and Mr. N. B. Akesson, for the loan of the heat-producing and measuring equipment.

until most of the plants had succumbed. The flat was watered only at the conclusion of each test, but at this time sufficient water was applied so that the subsoil remained too moist for drought to become a factor at any time, yet the surface layer of sand was dry enough on the second day after watering so that evaporation did not prevent the attainment of the desired temperature level. The 2-day interval gave ample time for the appearance of symptoms showing that a seedling had been fatally injured by a particular heat level.

With this method of conducting temperature tolerance tests, the first treatments probably influenced the effectivity of the subsequent ones by increasing the seedlings' heat resistance. This being the case, the procedure followed would seem very necessary if the results are to be used in connection with field problems, for it parallels the conditions to which seedlings are exposed in nature. Most seeds germinate in April and May, when the soil is moist, so that day by day as the soil dries out they are subjected to increasingly higher levels of soil-surface temperature.

The long flat was divided in half crosswise, and in each end a different method of soil heating was employed. Over one end was suspended a battery of four heating lamps which give off a high proportion of infra-red wave-lengths. For the other end of the flat a lead soil-heating cable, 6.5 mm. in diameter, was shaped into a grid, the segments of which extended back and forth between the rows of seedlings. This cable was put into position before the seeds were planted to avoid disturbance of the seedlings and was pressed down until about half the cable was below the soil surface. During the tests a thick wooden board was suspended 1 dm. above the lead cable, so that the seedlings in this end of the flat were screened from the heating lamps suspended over the other end.

Although different methods of soil heating were used during the tests, which were run simultaneously, the same temperature levels were maintained in both ends of the flat. The purpose of using two methods was to ascertain whether high surface temperatures per se, as maintained with the cable, are equally as important as high surface temperatures in conjunction with heating of the foliage as was the case under the lamps.

Despite all attempts to maintain uniformity of temperature over the flat, a certain amount of variation could be detected by moving the measuring junction of the thermocouple over the surface. Under the lamps the shade cast by the foliage of each seedling lowered the temperature a few degrees about the immediate base of the seedling. This problem was in part solved by thinning the stand of seedlings, as mentioned previously, and in part by using four heating lamps arranged in a row, so that the soil at the base of each seedling received heat rays from at least three angles. It is highly probable that some of the irregularities noted in seedling mortality may have been due to this factor, although the individual variations in tolerance are indeed great. Nevertheless, the differences among species proved to be so great, and the results obtained by the different methods of applying heat were so similar, that the experimental error appears to be negligible.

RESULTS.—The temperature tolerance tests (table 1) show marked differences among most of the species in their ability to tolerate high soil-surface temperatures, and these differences are correlated to a certain extent with the relative altitudinal distribution of the species: the

greater the tolerance of heat, the lower the natural occurrence of the tree. Fir and spruce, whose altitudinal ranges are so similar that they are generally considered dominants of the same (subalpine) forest belt, began to show injury between 40° and 45° C. Douglas fir and ponderosa pine resisted injury until temperatures of 55°–60° were attained, while piñon remained uninjured at temperatures of 70° C. Even in species relatively coloration of the lower 2 mm. of the stem) appeared simultaneously.

The temperature levels which proved lethal in this experiment vary considerably from values obtained by other investigators, and the latter are by no means in accord. Heretofore it has generally been thought that most coniferous trees of temperate North America have approximately the same degree of tolerance of soil-surface temperatures, and

TABLE 1

COMPARISON OF SURVIVAL OF SEEDLINGS DURING EXPOSURES TO INCREASINGLY HIGHER SOIL-SURFACE TEMPERATURE (° C.) LEVELS FOR 6-HOUR PERIODS

Heating equipment	Species	No. of seedlings	Cumulative mortality at different temperature levels (%)						
			40°	45°	50°	55°	60°	65°	70°
Heating lamps	Picea engelmannii	59	0	3	19	46	58	82	94
	Abies lasiocarpa	17	0	6	18	24	36	83	83
	Pseudotsuga taxifolia	17	0	0	0	6	6	24	36
	Pinus ponderosa	20	0	(10)*	(5)	0	(15)	25	30
	Pinus edulis	21	0	0	0	0	0	0	0
Soil cable	Picea engelmannii	62	0	11	48	82	82	†	.....
	Abies lasiocarpa	11	0	9	9	36	36	.....	.....
	Pseudotsuga taxifolia	15	0	0	0	7	27	.....	.....
	Pinus ponderosa	9	0	0	0	11	11	.....	.....
	Pinus edulis	21	0	0	0	0	0	.....	.....

* Mortality due to damping-off rather than to heat treatment and therefore not included with cumulative mortality due to heat. Of all the species studied this pine has consistently proved the most susceptible to damping-off under greenhouse conditions.

† The soil cable proved inadequate for obtaining soil-surface temperatures above 60° C.

sensitive to heat a few seedlings were able to develop extraordinary resistance. For example, 17 per cent of the subalpine fir seedlings withstood the highest temperatures employed.

Nearly identical results were obtained with the two methods of heating, a fact which strongly suggests that the injurious effects of insolation are truly the result of heat injury at the base of the stem rather than the result of excessive absorption of heat by the foliage. Further evidence substantiating this conclusion is that with both methods of heating, symptoms of heat injury (a grayish dis-

various investigators have reported injurious temperatures as beginning at 50° C. (24), 52° C. (14), 60° C. (14), and 62° C. (13). In the writer's experiments injury began at 40°–45° for certain species, while one species apparently remained uninjured at 70° C. Using seedlings 39–58 days old, ROESER (21) found injury to begin in Engelmann spruce at 51° C., in Douglas fir at 53°, and in ponderosa pine at 50°. According to his results there is little difference among the species as to their temperature tolerance, and this difference is not related to altitudinal range. Possibly some of the di-

versity between his results and the writer's may be attributed to differences in technique; with ROESER's technique the seedlings were heated in a manner which differed far more than the writer's from natural conditions.

It is also likely that much of the discrepancy in results with these tests is due to differences in methods of measuring surface temperatures. Soil "surface" temperatures have been variously measured—by laying the sensitive element of the thermometer (commonly 5–27 mm. in diameter) on the surface, by partly imbedding it, or by burying it just deep enough so that its surface is thinly covered with soil. Such methods lead to widely different conclusions, for under strong insolation there is an extremely sharp temperature gradient in both directions from the soil surface. Using a thermocouple, the writer has found that under strong insolation the air 5 mm. above and the soil 5 mm. below the soil surface may be 15.0° and 5.3° C. cooler, respectively, than the surface itself. In view of this condition it is difficult to see how a thermometer with a bulb 5 mm. in diameter could be used to measure true surface temperatures accurately. Certainly no temperature is as significant as the maximum, and that is obtained by the very surface layer of particles. The thermocouple is the only instrument adequate for measuring such temperatures, since the sensitive part, the juncture of two fine wires, is less than 1 mm. in diameter. It may be concluded that possibly many of the data quoted as critical soil-surface temperatures for seedling survival are hardly more than crude approximations.

Among the species employed in this series of studies, those with thin-stemmed seedlings grow at high elevations, while the pines, especially piñon, which have relatively thick stems, grow at low elevations. It might appear that differences in tolerance of high soil-surface temperatures, and hence lower altitudinal limits, could be explained on the basis of relative thickness of insulating tissues in the vicinity of the soil surface. However, close analysis of the survival data in relation to stem anatomy, together with evidence provided by the interpretation of species distribution, militate strongly against this hypothesis.

In spruce, subalpine fir, and Douglas fir all bark tissues were still living at the time the experiments were performed. Cross sections of the stems just above the soil surface showed that subalpine fir and Douglas fir had approximately equal stem diameters and bark thicknesses, yet these species showed distinctly different abilities to tolerate hot soil surfaces. On the other hand, subalpine fir seedlings exceeded those of spruce in stem thickness and bark thickness, but both species exhibited the same degree of heat resistance. Further evidence that stem morphology of seedlings is not very well correlated with altitudinal distribution is provided by the fact that the thick-stemmed white-bark pine (*Pinus albicaulis*) is limited to high elevations, while the thin-stemmed junipers are best represented near lower timberline.

EVALUATION OF RESULTS.—It was not feasible in the field to ascertain directly whether or not the maximal soil-surface temperatures just below the natural limits of distribution of each species are everywhere in excess of its tolerance, as found in the greenhouse tests. A priori, such tests seem unnecessary for three reasons.

First, it is well known that even a thin shade will keep the temperature of the soil surface lower than that of the air, for the air is the source of practically all the

heat which a shaded surface may come to possess. KORSTIAN and FETHEROLF (15) found that high soil-surface temperatures caused lesions at the ground line in seedlings of Engelmann spruce when planted below their natural elevational limits in Utah, but they also observed that even a slight shading of the soil at the base of the stem, caused by an inclination of the seedling's axis toward the south, greatly reduced mortality from this cause. As PEARSON (19) pointed out, if maximal temperatures were the factor which prevents Engelmann spruce from advancing downslope into the territory occupied only by Douglas fir, one would expect to find spruce seedlings becoming established under the dense canopies of young Douglas fir stands, or at least in shaded nooks formed by the fortuitous juxtaposition of trunks, fallen logs, rocks, etc. The fact that there is an abundance of micro-habitats in each forest zone which are perpetually sheltered from direct sunlight seems to constitute excellent evidence that none of the species at their lower altitudinal limits have attained a point below which they cannot extend because of an intolerable degree of heat.

A second type of evidence which militates strongly against the temperature hypothesis is the fact that the School of Forestry of the University of Idaho maintains a nursery in which seedlings of *Pinus monophylla*, ponderosa pine, Douglas fir, and subalpine fir may be grown entirely without shade (20). The site of this nursery is on the basal plain below lower timberline, and the midsummer season here is characterized by strong insolation. The only special care which the seedlings require is frequent sprinkling, for the months of July and August have exceedingly low rainfall.

Lastly, the laboratory tests revealed no significant difference in the temperature tolerance of ponderosa pine and Douglas fir seedlings, yet throughout those parts of the Rockies where both species occur there is a distinct difference in their altitudinal ranges, the pine invariably extending to a lower altitude than the fir. If the fir is equally as resistant as the pine to high temperature, clearly some factor other than surface temperatures must keep the fir from extending downslope into territory occupied by the pine.

It has been demonstrated that in different parts of the ranges of ponderosa pine and Douglas fir the species are represented by races differing in their physiological characteristics, and the same will undoubtedly be found true for other Rocky Mountain trees. Therefore the practice of comparing species representative of the various zones by using seed collected in different parts of the Rockies might be questioned. In northern Idaho at least it would be practically impossible to use seed collected from different zones in the same region unless seed collections were begun several years in advance of the study, for the years when different species produce seed seldom coincide. On the other hand, the consistency of zonal sequences and of all the experimental results obtained in this study provide strong evidence that the procedure followed is justified.

PHASES OF DROUGHT

An unfavorable water balance in plants may be brought about by excessive transpiration, even though soil-moisture conditions are favorable. On the other hand, a degree of desiccation equally as dangerous can result from inadequate soil moisture at times when the transpiration rate is not especially rapid. An attempt has been made to test sepa-

rately the degree of tolerance which conifer seedlings have for these two aspects of drought. Since both atmospheric and soil drought increase in intensity downslope, any differences among the species as to their relative tolerance of these factors might prove significant in differentiating their altitudinal range limits.

Relative tolerance of atmospheric drought

Methods.—The next spring after the temperature tolerance experiments were carried out, another crop of seedlings was grown in the same flat of soil. The species used in this study, together with the seed source, are listed in order of their zonal relationships:

Subalpine fir—San Isabel National Forest, Colorado
Engelmann spruce—San Isabel National Forest, Colorado
Western arborvitae (*Thuja plicata*)—Latah County, Idaho
Douglas fir—Latah County, Idaho
Ponderosa pine—Colorado
Piñon—Colorado

In this experiment the seeds were planted in rows at right angles to the length of the long flat, the rows of mixed species being 5 cm. apart. When the seedlings were 75 days old, drought experiments were begun.

About 24 hours prior to each test the flat was watered heavily, and by the time the test was begun the surface of the sand was dry, although the soil below was well above the field capacity. Desiccation was prevented from extending much below the very surface by covering the soil between the rows of seedlings with strips of glass. After each test, examination showed that this method was adequate to insure favorable level of soil moisture, and therefore any drought effects could be attributed entirely to atmospheric conditions.

During each test a piece of heavy wrapping paper was bent over the flat and secured to both sides with thumbtacks in such a manner that a tunnel, open at both ends, remained above the soil. The diameter of the open ends was adjusted to accommodate an electric desiccating fan, which sent a current of warm dry air through the tunnel across the foliage of the seedlings. Midway during each test the fan was moved from one end of the flat to the other, so that any effects due to differences in the distances between the fan and rows of seedlings would be partly offset, and the results seemed to indicate that desiccation was approximately uniform. An atmometer with the sphere at the end of the tunnel opposite the fan measured the evaporative rates during the tests. These rates were probably a little higher than this instrument recorded, since it was always located at the end of the tunnel farthest from the fan and there the relative humidity was undoubtedly higher than anywhere else along the tunnel, owing to the continual addition of moisture by the air current as it passed through the foliage of the seedlings as well as over the very small percentage of the soil surface not conveniently covered with the glass.

On the first test the seedlings were subjected for 4 hours to an artificial breeze with a mean evaporative rate of 10.3 ml. per hour. It was planned thereafter to make a series of tests at intervals of a few days, each time increasing the duration of treatment, to determine the degree of drought which the different species could endure. Duration tests of 6, 8, and 10 hours were made, during which the mean rates of evaporation were 9.4, 9.9, and 8.5 ml. per hour, respectively. During these tests sufficient measurements of air

temperature were made within the wind tunnel so that it was certain that the temperature remained well below the critical levels as found in the preceding experiment, and the fact that survival was nearly 100 per cent proves that this factor was not important. The fifth test, which lasted for 12 hours, was conducted on a day during which insolation became so exceptionally strong that practically all seedlings succumbed, and there was no assurance but that high temperature played as important a role as the excessively high evaporation rate. However, the duration and intensity of the previous tests had already increased to such a degree that the most extreme conditions of atmospheric drought encountered by these seedlings in their natural habitats had been exceeded, so that the data obtained prior to the final test are sufficient to answer the question which prompted the tests.

RESULTS.—During drought tests of 6–10 hours, very few seedlings succumbed (table 2), and none of these died as a result of the two most severe tests. The survival and vigor of none of the species was impaired by a 10-hour exposure to a current of warm (not critically hot) air which produced a mean evaporative rate of 8.5 ml. per hour.

EVALUATION OF RESULTS.—In all cases the degree of artificial drought maintained in the preceding experiments was extremely severe in comparison with field conditions in the Rocky Mountain region. On a hot midsummer day at Moscow, Idaho, which is situated below lower timberline, the evaporation rate at 20 cm. above dry barren ground in full sunlight has measured but 3 ml. per hour for the 12-hour period centering about noon. The maximum hourly evaporation rate which CALDWELL (6) obtained with cylindrical atmometers in the desert near Tucson, far below lower timberline, was only 6.1 ml. per hour. With the lowest mean hourly evaporation rate 8.5 ml. per hour in these experiments, it is apparent that all the coniferous seedlings tested were able to survive atmospheric drought far in excess of what most of the species normally encounter in nature. No importance can therefore be assigned this aspect of the environmental water balance per se in determining the altitude down to which each species can extend its range.

TABLE 2

COMPARATIVE ABILITIES OF SEEDLINGS TO ENDURE ARTIFICIALLY PRODUCED CONDITIONS OF ATMOSPHERIC DROUGHT

Duration of period (hours)	Mean hourly evaporation (ml.)*	Mortality (%)					
		Pinus edulis	Pinus ponderosa	Pseudotsuga taxifolia	Thuja plicata	Picea engelmannii	Abies lasiocarpa
4	10.3	0	0	0	2	4	4
6	9.4	0	0	2	3	2	0
8	9.9	0	0	0	0	0	0
10	8.5	0	0	0	0	0	0
12	12.7	87	92	81	97	94	96
Total no. of seedlings..		46	50	40	112	47	47

* From standardized spherical atmometer.

Additional data which show the relative insignificance of the evaporative rate, at least in so far as it operates directly on the seedlings, have been obtained by measuring evaporation in five vegetation zones (from the prairie to the Engelmann spruce zone) in the vicinity of Moscow (8). These field data show that there is no important difference in the evaporation rates in climax associations from one zone to the next during the season most critical for seedling survival. This, of course, does not preclude the likelihood that the slight increase in evaporation rate downslope does not have an indirect effect upon seedling survival through its

influence in drying the surface layers of soil.

The conclusion already reached, that —among other species—Douglas fir seedlings are able to withstand a much drier air than they ever encounter within the natural range of the species, appears to be at variance with PEARSON'S conclusions (17). In Arizona he found that Douglas fir seedlings succeed in the shelter of aspen cover but not in adjacent forest openings. Soil moisture was favorable in both habitats, whereas the evaporative rate was at times as much as 90 per cent greater in the open, and it was concluded that the evaporative rate was the controlling factor. This suggestion may hold true for seedlings younger than those used in the present drought tests, or possibly some other factor not measured may have differed sufficiently to bring about different mortality rates in the two habitats which PEARSON compared.

RELATIVE TOLERANCE OF SOIL DROUGHT

Obviously, any variation on the part of the seedlings of different species of conifers to endure dry soil might be of considerable significance in setting different lower altitudinal limits, for both the frequency and the intensity of drought periods increase downslope.

METHODS.—A few seeds of each of the species used in the preceding experiment were planted in each of eighteen metal containers 12 cm. in diameter and 8 cm. in depth. These were coated inside with asphaltum and were crowded together on the greenhouse bench so that excessive soil heating through direct insolation upon the outside of the cans was avoided.

Although conifer seedlings quickly become too cutinized to show when the wilting point is reached, it has been demonstrated that the detection of the attainment of the wilting percentage in such plants is easily accomplished in an indirect manner by interplanting species with more delicate leaf structure (9), since the wilting percentage is essentially the same for all species. When the conifer seedlings were 70 days old, sixteen grains of wheat were sown in each pot. By the time the wheat shoots were about 1 dm. tall their extensive and finely divided root systems had spread throughout the soil mass, and at this time irrigation was suspended so that the plants (coniferous and wheat seedlings) thenceforth gradually reduced the soil moisture to the wilting percentage, as indicated by the appearance of the wheat leaves. Through this use of wheat seedlings it was hoped to overcome the problem of achieving soil drought simultaneously for the deep-rooted pines and the shallow-rooted spruce and fir. The soil mass was so thoroughly ramified by the wheat root systems that it seems safe to assume that its moisture content was reduced rather uniformly and the wilting percentage attained at all horizons at approximately the same time.

Note was taken of the time at which most of the wheat leaves in each pot wilted permanently. Several of the pots were allowed to remain with the soil moisture at or below the wilting percentage for 2 days, then they were watered and the seedlings observed for a time to determine the effects, if any, of the short interval of drought on the vigor of the shoots of the various conifer seedlings rooted in the pot. A second group of pots was allowed to remain in the desiccated condition for 4 days, etc., until a series of data for drought periods up to 10 days was accumulated. This experiment was performed twice, with essentially identical results; the more complete set of data is presented in table 3.

RESULTS.—There were definite differences among species with respect to the abilities of their seedlings to survive approximately identical conditions of soil drought. In general, the conclusion seems warranted that those species characteristic of relatively high elevations can tolerate very little desiccation, whereas those which normally grow at low altitudes can survive periods of soil drought several times longer without apparent injury. However, other tests not included in table 3 indicate that even the drought-resistant seedlings of piñon cannot endure dry soil for more than about 12 days.

PEARSON (18) found that the more mesophytic species of conifers "possess a surprising degree of resistance to transpiration when the water supply is reduced to a dangerous level." This characteristic may well explain the greater tolerance of soil drought in these species, in that the ability greatly to decrease water loss postpones the attainment of a fatal degree of protoplasmic dehydration.

EVALUATION OF RESULTS.—In all forest zones In the Rockies, the soil-moisture content is favorable in the spring and early summer, when tree seedlings germinate, but decreases progressively during the summer. Both PEARSON (18) and HAIG *et al.* (11) have emphasized the importance of the rate of elongation of the roots of seedlings with respect to this progressive desiccation from the soil surface downward. Growth water remains available to the seedlings only so long as the distal extremities of their root systems keep ahead of the downward extension of the dry zone. When due consideration is given to the well-known differences among the species as to rate of penetration in depth of their root systems, the significance of the differences in physiology as here reported is greatly enhanced. Those species shown to be most tolerant of dry soil likewise have the greatest rates of root elongation; thus the two characteristics have cumulative value in regard to survival in dry climates. For example, the tap root of piñon, which has the most rapid rate of elongation and consequently runs the least risk of being overtaken by progressive soil desiccation, is at the same time the most able to endure such a dangerous condition until it can be alleviated by precipitation.

TABLE 3

COMPARATIVE SURVIVAL OF SEEDLINGS WHEN SOIL-MOISTURE CONTENT WAS BELOW WILTING COEFFICIENT, 5 DAYS AFTER IRRIGATION HAD BEEN RESUMED

No. of days of soil drought	Mortality (%)					
	Pinus edulis	Pinus ponderosa	Pseudotsuga taxifolia	Thuja plicata	Picea engelmannii	Abies lasiocarpa
2.....	0	3	0	.....	33	0
4.....	0	0	50	100	50	100
6.....	0	19	80	100	100	100
8.....	0	50	100	100	100	100
10.....	0	100	100	100	100	100

Field studies have invariably shown that the intensity of soil drought increases downslope in the Rockies. WEAVER (26), HANSON (12), and WHITFIELD (27) have presented limited but consistent data which demonstrate this point. PEARSON (19), who has made the most complete series of studies of this factor to date, stated that in the ponderosa-pine zone in the southern Rockies at "depths of 12 inches or less, complete or nearly complete exhaustion of growth water may usually be expected in the latter part of June. All information at hand indicates that far more extended depletion

is the rule in the piñon-juniper type. In the Douglas fir and Engelmann spruce types, droughts during the main growing season must be exceedingly rare." Periodic measurements of soil moisture in the northern Rockies (8) bear out these conclusions. Thus the environmental measurements appear to furnish another source of indirect evidence concerning the dominant role played by the moisture factor, for approximately equal degrees of drought recur season after season in various zones in the southern Rockies, and there appears to be close similarity in the manner with which differing degrees of drought are correlated with the same forest types in the northern and southern Rockies. The importance of moisture as a governing factor in setting lower limits of altitudinal distribution in the Rockies is still no more than a theory, but there has accumulated an impressive body of field data and physiological tests to substantiate it.

Summary

1. The differing abilities displayed by various Rocky Mountain coniferous trees to extend their ranges downslope into altitudes of increasingly hotter and drier climate has been attributed by some investigators to corresponding differences in the ability of their seedlings to tolerate heat, while others explain the same phenomenon on the basis of drought. In an effort to determine which of these is correct, greenhouse studies were made in which seedlings of trees with different altitudinal ranges have been compared in respect to their tolerance of high soil-surface temperatures and their tolerance of atmospheric and soil drought.

2. In general, it was found that the lower the altitudinal distribution of a species the greater the tolerance of its seedlings for high soil-surface temperatures. However, this factor apparently cannot explain the lower altitudinal limits, since (*a*) the maximum temperature level which each species can tolerate well exceeds the maximum temperatures obtaining in numerous micro-habitats below its altitudinal range; (*b*) a number of species, including subalpine fir, can be grown far below their natural lower limits without making compensation for the higher soil-surface temperatures there; and (*c*) the correlation between altitudinal distribution and temperature tolerance is not absolute, ponderosa pine having no greater temperature tolerance than Douglas fir, although it extends to distinctly lower altitudes.

3. When supplied with abundant soil moisture, even the subalpine trees can tolerate an intensity of atmospheric drought far in excess of that to which they are subjected under natural conditions. Furthermore, all species, regardless of altitudinal range, have approximately equal resistance to atmospheric drought. This aspect of drought therefore is apparently of no direct importance in determining the lower limits of the trees.

4. A distinct variation exists in the length of time over which the seedlings of different species can tolerate a lack of growth water; in general, the lower the altitudinal range the longer the period of soil drought which can be endured. Since all researches to date have indicated that the intensity and duration of drought increase downslope, this factor appears to offer at least a partial explanation of the differences in lower range limits. The significance of this difference among the species to endure soil drought is greatly magnified by the fact that the species of low altitude have the most rapid rates of root penetration. Not only are they bet-

ter able to endure soil drought, but they are more likely to escape its influence.

5. The accuracy with which uniformity of environmental conditions can be maintained in different parts of the same culture varies inversely with the number of experimental plants used, and therefore conclusions based upon a few carefully tended plants are at least equally as valuable as greater numbers of plants but with conditions less uniform. In the temperature tolerance tests in which the fewest numbers of seedlings were used, the same conclusions may be drawn from either half of the lot of seedlings.

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EFFECTS OF NUTRIENT, PHOTOPERIOD, AND NIGHT TEMPERATURE ON THE DEVELOPMENT OF GUAYULE SEEDS

M. R. WHITEHEAD[1] AND JOHN W. MITCHELL[2]

Introduction

Although the rubber-producing plant guayule, *Parthenium argentatum*, produces abundant seeds when grown under favorable conditions, a relatively large number of these contain immature embryos. Improvement in the quality of the seeds produced would be of value from a practical standpoint, since the number gathered could be reduced without diminishing the yield; and from the standpoint of an experimental breeding program, it would make possible the more rapid multiplication of desirable strains.

Experiments were undertaken to test the effect of several environmental factors on the quantity and quality of the seeds, as well as on the amount of vegetative growth and rubber produced by the plant. The results reported here concern seed production when the plants were (*a*) supplied with various nutrients, (*b*) subjected to different night temperatures, and (*c*) grown under different photoperiods.

Investigation

Nutrient supply

Methods.—At the time the present experiments were undertaken, relatively little work had been done with respect to the nutrient requirements of guayule. Preliminary efforts were therefore directed toward a general study of the effect of different types of nutrient solutions on growth of the plant and its rate of seed production, rather than toward a detailed study of the influence of any one specific element.

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Two series of solutions were used, as described by Hamner (4). In the first series the cations Ca, K, and Mg varied in concentration while the anions remained essentially constant, and in the second series the anions N, P, and S varied while the cations remained constant. Since the object was to select a nutrient solution which would result in the greatest amount of growth, together with a maximum rate of seed and rubber production, it was not considered necessary to test solutions which did not contain all the essential elements.

Three stock solutions were used to prepare nutrient solutions in which the anions varied. Stock solution A consisted of 0.0037 mol. KNO_3, 0.0037 mol. $Mg(NO_3)_2$, and 0.0049 mol. $Ca(NO_3)_2$. Solution B consisted of 0.0037 mol. K_2SO_4, 0.0048 $CaSO_4$, and 0.0037 mol. $MgSO_4$. Solution C consisted of 0.003 mol. $Ca(H_2PO_4)_2$, 0.0023 mol. $MgHPO_4$, and 0.0037 mol. KH_2PO_4. Sufficient phosphate was added as H_3PO_4 to make the phosphate ion in solution C equivalent to that in a 0.0037 mol. solution of $MgHPO_4$.

In a similar manner three stock solutions were used to prepare nutrients which varied with respect to the cations. Stock solution D contained 0.0037 mol. $Mg(NO_3)_2$, 0.0037 mol. $MgSO_4$, and 0.0023 mol. $MgHPO_4$. Phosphate was added as before to raise the concentration of this ion in D to that of a 0.0037 mol. solution of $MgHPO_4$. Solution E contained 0.0037 mol. KNO_3, 0.0037 K_2SO_4, and 0.0037 KH_2PO_4. Solution F contained 0.0049 mol. $Ca(NO_3)_2$, 0.0049 mol. $CaSO_4$, and 0.0033 mol. $Ca(HPO_4)_2$. All solutions were made up in tap water.

By adding together the required

amounts of these stock solutions, it was possible to make nutrient solutions which varied with respect to their anion or cation content, while the ionic concentration of the cations remained essentially the same (table 1). However, when the concentrations of the elements nitrogen, phosphorus, and sulphur were expressed on a part-per-million basis, they necessarily varied from 78 to 118, 131 to 175, and 124 to 144 p.p.m., respectively. It was likewise possible to make a corresponding series of solutions which varied a desired amount with respect to their cation content, while the anion concentration remained essentially constant. Expressed on a part-per-million basis, calcium, magnesium, and potassium in the latter series varied from 154 to 184, 66 to 84, and 168 to 240 p.p.m., respectively. Boron, zinc, copper, and manganese were added at the rate of 0.5 p.p.m. in the form of boric acid, zinc chloride, copper chloride, and manganese chloride. Iron was added as ferric citrate at the rate of 0.3 p.p.m.

One thousand uniform transplants, variety 593, which had been grown in a nursery in California, were selected for size and uniformity. These plants were of a size commonly used for field planting. They were planted individually, during the first week of July, in a gravel-sand mixture contained in glazed earthenware crocks of 2- and 3-gallon capacity. The mixture contained 27% by weight of coarse sand which passed an $\frac{1}{8}$-inch screen, 31% gravel which passed a $\frac{1}{4}$-inch screen, and 42% gravel which failed to pass through a $\frac{1}{4}$-inch screen. The crocks were rounded on the bottom and provided with a 1-inch hole for drainage. This opening was covered with a piece of bronze screen to prevent the loss of sand and gravel. The crocks were supported on bricks so that they were approximately 2 inches above the ground, or above the surface of gravel on a bench in the case of the greenhouse experiments. This allowed free drainage and prevented the roots from growing out of the pots. Sufficient nutrient solution was applied to flush the pots three times weekly. The sand-gravel mixture was kept moist during the intervening time by light applications of tap water, which were applied carefully so as not to flush the nutrients from the crocks.

TABLE 1

PROPORTIONS OF STOCK SOLUTIONS USED IN PREPARATION OF NUTRIENT SOLUTIONS

Solution	Stock solutions			Solution	Stock solutions		
	A	B	C		D	E	F
1	$\frac{2}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	11	$\frac{2}{3}$	$\frac{1}{6}$	$\frac{1}{6}$
2	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{3}$	12	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{3}$
3	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$	13	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$
4	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{2}{3}$	14	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{2}{3}$
5	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{6}$	15	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{6}$
6	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	16	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$
7	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$	17	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$
8	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$	18	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$
9	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$	19	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$
10	$\frac{1}{6}$	$\frac{2}{3}$	$\frac{1}{6}$	20	$\frac{1}{6}$	$\frac{2}{3}$	$\frac{1}{6}$

The seedlings were divided into two groups of equal numbers. One group was placed outdoors during July, August, September, and the first half of October, so that the plants were exposed to direct light and prevailing temperatures during this period. The remaining group was placed in a greenhouse. In both groups treatments were arranged as randomized blocks. Seven replications were used in the case of the greenhouse experiment and five in the case of the experiment conducted outside.

Plants grown in the open were subjected to direct light which reached an intensity of more than 10,000 foot-candles on very clear days, as measured

by a Weston light meter. Night temperatures during July and August varied between 60° and 75°, while day temperatures sometimes reached 90°–98° F. During September and early October night temperatures fell as low as 30°–40°, and day temperatures seldom were more than 70°–80°. In October, those plants which had grown outside during the summer were moved into the greenhouse, where they were subjected during the fall and winter months to a day temperature of 65°–75° and a night temperature of 45°–55° F.

Plants grown in the greenhouse were subjected to light which filtered through double-strength glass. The intensity was reduced by approximately 30% below that of direct light, as measured by a Weston light meter. Night temperatures in the greenhouse during July and August seldom fell below 70°, and day temperatures usually ranged between 85° and 95° and occasionally reached 105°–110° F. During September and early October, day temperatures in the greenhouse still ranged 5°–15° above outside temperatures, although night temperatures were more nearly the same. The plants which had been subjected to relatively high temperatures under greenhouse conditions during the summer months were also grown at relatively high temperature levels during the fall and winter months; the day temperatures were 70°–80° and the night temperatures 60°–70° F.

The plants began to flower during the latter part of July, and the first seed collections were made the middle of August. The flowering season continued during the remainder of August and all of September but became greatly reduced with the onset of cooler weather during October. Practically no flowers developed after the middle of October.

In collecting samples, seeds were gathered at intervals of 2–5 days, when ripe. They were separated from the chaff and stored air-dry at room temperature in groups, which in the end represented the total number of seeds produced during the summer and fall seasons by the respective replications of each treatment.

The expression "seed yield" is used here to mean the dry weight of the total number of seeds produced by a plant during a given period of time. The seed, pistillate floral parts, and attached disk flowers (2) were included in the seed weights because of difficulty of separation. Two methods of measurement were employed to determine the quality of the seeds produced. The one most generally used consisted of determining the percentage of seeds within a given lot which could be classified as heavy, and which for the most part contained seeds with mature embryos. The other method consisted of testing the heavy seeds for germination.

Relative to estimating the percentage of heavy seed, a satisfactory and rapid separation of those with mature from those with immature embryos was accomplished by means of the specific gravity method, using Skellysolve[3] fraction F (fig. 1). The relatively heavy seeds included most of those with mature embryos, while the relatively light seeds most of those with immature embryos.

For the purpose of measuring percentage germination, the light seeds were discarded and only the heavy ones tested. These were first soaked in tap water overnight at room temperature. The water was then drained off and a 4 per cent mixture of sodium hypochlorite

[3] Miss Alice M. Andersen, Bureau of Food Distribution Administration, first used Skellysolve to separate guayule seeds.

added for a period of 20 minutes. The seeds were then washed with water two to three times to remove the hypochlorite. They were then placed on moist filter paper in petri dishes, and these were alternately placed at 20° C. for 16 hours and then at 30° C. for 8 hours. The dishes were kept in continuous darkness, except when being transferred from one temperature to the other.

Germination was considered to be that stage of development at which the radical emerged from the seed coat. The rate of growth subsequent to this stage was variable, as some plants grew vigorously while others grew more slowly. During the first 2–3 days' growth the seedlings were classified as good or poor, depending on the rate at which the radical was extended. In this way it was possible to measure the percentage of seedlings which grew vigorously during the very early stages of development.

Two hundred and twenty-five seeds were tested for germination in the case of the majority of the nutrient treatments. Plants of a few treatments failed to produce sufficient seeds for adequate germination tests and therefore a statistical analysis of germination data was not attempted.

RESULTS.—Figure 2 compares the amount and quality of seeds produced by plants grown on solutions varying with respect to anions. Plants supplied with a relatively high level of nitrogen (solution 1) produced approximately nine times as much total yield of seeds by weight as the average amount produced by plants supplied with relatively low levels of nitrogen (solutions 4, 7, 9, 10). In general, an increase in the amount of nitrogen above 48 p.p.m. was associated with increase in the weight of seeds produced, irrespective of variations in the sulphur and phosphorus content of the solutions. Thus plants watered with solutions 4, 7, 9, and 10, which all contained a low level of nitrogen, produced relatively few seeds. No significant differences in seed production were noted between plants watered with these four solutions, although the concentration of sulphur varied from 65 to 260 p.p.m. and that of phosphorus from 72 to 288 p.p.m. Solutions 3, 6, and 8 contained a somewhat higher concentration of the nitrate ion, and the plants watered with them produced a significantly greater number of seeds than did those of the previous series. Again there was no significant difference in weight of seed produced due to the different levels of sulphur and phosphorus used in this second series. The use of solutions 2 and 5 resulted in a further increase in seed production, and the increase was associated with an increase in the concentration of nitrate ion used. As in the previous series, varia-

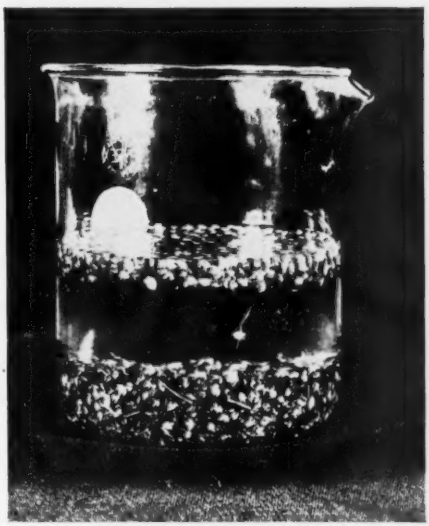
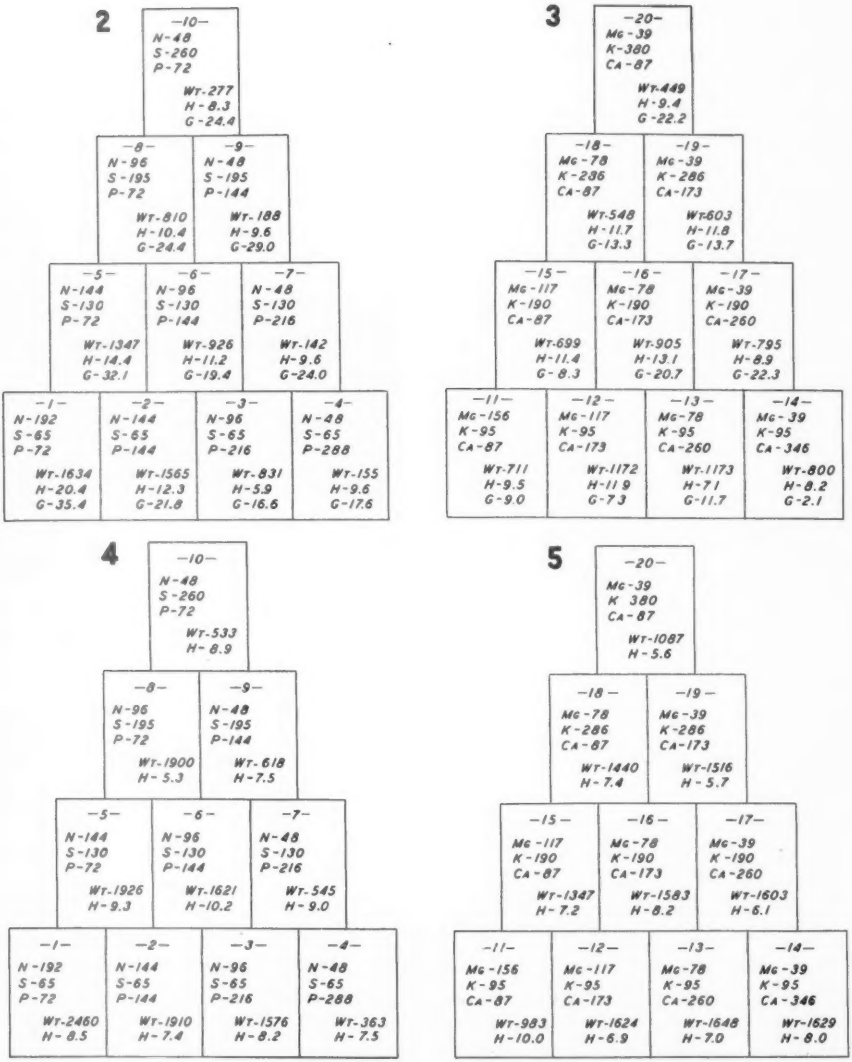


FIG. 1.—Method of separating light from heavy seeds, using Skellysolve.



FIGS. 2–5.—Seed production as influenced by nutrient solutions: Figs. 2, 3, plants grown outdoors; figs. 4, 5, grown in greenhouse. (*wt*, average weight of seeds collected during 2 months from each replication, four plants; 236 mg. difference required for statistical significance in seed weight of 1:19 in figs. 2 and 3. *H*, percentage of heavy seeds; difference of 5.5 per cent required for statistical significance of 1:19. *G*, percentage germination of heavy seeds.) Figs. 2, 4, solution varied with respect to N, nitrogen; S, sulphur; P, phosphorus; figs. 3, 5, with respect to Mg, magnesium; K, potassium; Ca, calcium.

tions in the amount of sulphur and phosphorus were not associated with significant variations in the weight of seeds produced. From this it appears that the range of concentrations in the case of the sulphate and phosphate ions was not critical, but that an increase from 48 to 192 p.p.m. of nitrogen in the nutrient was associated with a marked increase in the amount of seeds.

Essentially the same type of response was observed with respect to the quality of seeds produced by plants grown on solutions containing different amounts of the anions. An increase in nitrogen from the lowest level (solutions 4, 7, 9, 10) to the highest (solution 1) was associated with substantial increase in the percentage of seeds with mature embryos, that is, the percentage of heavy seeds. Seeds from plants grown on a solution containing a relatively large amount of nitrogen also gave the highest percentage germination. The concentrations of sulphur and phosphorus used were not critical with respect to seed quality.

Figure 3 shows the effect of different concentrations of cations on the amount of seed produced. A solution containing a relatively low concentration of magnesium and calcium and a high level of potassium (solution 20) did not favor seed production. With increasing amounts of magnesium and calcium there was a corresponding increase in the amount of seed, which reached a maximum in the case of plants watered with solutions containing 78–117 p.p.m. of magnesium and 173–260 p.p.m. of calcium (solutions 12 and 13). In these solutions potassium was at the lower limit of the concentration range for this element, but at this level there was apparently an ample supply of potassium. A high level of calcium together with a low level of magnesium was associated with reduced seed production (solution 14). The reverse of this combination (high magnesium and low calcium) was also associated with reduced seed production (solution 11), and plants grown on this solution showed symptoms of injury which were apparent mainly in the leaves.

A relatively high percentage of heavy seeds was produced by plants supplied with a moderate amount of magnesium and calcium and a relatively high level of potassium (solution 16), although a somewhat greater total weight of seeds was obtained through the use of solutions containing essentially the same levels of magnesium and calcium but a lower level of potassium.

When different concentrations of anions were supplied to plants grown under greenhouse conditions (fig. 4), the responses with respect to the amount of seeds produced were similar to those of plants treated in the same way but grown in the open. Increasing amounts of nitrogen supplied to the plants, 48–192 p.p.m., resulted in a corresponding increase in the weight of seeds produced (cf. 4, 7, 9, 10 with solution 1). A solution which contained a low sulphur level, together with low nitrogen and high phosphorus, was somewhat less favorable than were other solutions low in nitrogen but containing somewhat more sulphur (cf. solution 4 with 7, 9, and 10). In general, the concentrations of sulphur and phosphorus were less effective in altering seed production than were the concentrations of nitrogen.

Considering the utilization of nitrogen, plants grown outside and supplied with 192 p.p.m. of nitrogen produced approximately nine times more seeds than did other plants given solutions containing 48 p.p.m. of nitrogen (cf. solution 1 with the average of 4, 7, 9,

10; fig. 2). In contrast, plants grown in the greenhouse and given 192 p.p.m. of nitrogen produced only 4.8 times more seeds than did others watered with solutions containing 48 p.p.m. of nitrogen (fig. 4).

Under greenhouse conditions, as in the case of plants grown outside, those that received the highest level of magnesium together with a low level of calcium and potassium (solution 11) showed symptoms of injury, especially in the leaves, and these plants produced relatively few seeds (fig. 5).

Greenhouse plants responded somewhat differently from those grown outside, in that their rate of seed production was not inhibited by application of a solution containing 39 p.p.m. magnesium and a relatively high level of calcium. Also, plants grown under the reduced intensity of light and relatively high temperatures prevailing in the greenhouse produced a greater total weight of seeds in the case of most treatments than did those grown outdoors; but they were of poor quality with respect to the percentage containing mature embryos.

Although the nutrient treatments had an effect on the percentage germination of seeds from plants grown outdoors, they did not obviously affect the rate of growth of the seedlings during a 2-week period subsequent to germination. Since no significant differences between treatments were noted in the number of vigorously growing seedlings, data from these observations are not presented.

Photoperiod

Methods.—The object of this experiment was to observe the effect of the length of the daily period of illumination on the number and quality of seeds produced. In general, seedlings were exposed to daily periods of illumination of different lengths, the photoperiodic treatment being applied as soon after germination as feasible. The effect on flower initiation and development and on seed production was observed during several months of treatment and for some time thereafter.

Seeds of variety 406 were germinated in petri dishes as previously described. When the radicals were approximately $\frac{1}{4}$ inch long, selected seedlings were planted in soil and grown for 2 weeks under conditions prevailing in the greenhouse during June. At the end of this time the tops extended about 1 inch above the surface of the soil. Ninety uniform plants were selected and divided into six groups of equal numbers, and each group was placed on a truck which could be moved from the natural light of the greenhouse to rooms where artificial light was the only source of illumination. The number of plants used was limited by the capacity of the trucks.

Photoperiodic treatments were chosen which corresponded in length to natural daily periods of illumination prevailing in different latitudes in the United States suited to the growing of guayule. In addition, two other treatments were added. In one, the plants were subjected to day lengths prevailing at Beltsville, Maryland, during the period between July and January; in the other, supplementary light was added so that the plants were exposed to a continuous period of illumination.

For the purpose of comparing some of the treatments, it was thought desirable to subject them to the same amount of natural light, namely, a basic period of 10 hours, so that the plants would have the same opportunity to synthesize food material. To extend the daily periods of illumination, this basic period was supplemented by means of light from a

white fluorescent lamp. The supplementary light was of low intensity (20 foot-candles) so as to induce a photoperiodic response without bringing about an appreciable amount of photosynthesis.

Treatments were applied from July 1 until November 3, when the plants were removed from the trucks and placed on a greenhouse bench where they all received natural illumination under the same conditions. Photoperiodic responses were observed from September to January, during a major part of which time the plants were in bloom.

RESULTS.—Under the conditions used, a relatively long daily period of illumination favored the initiation of flowers, the production of seeds, and to some extent lengthened the flowering season (table 2). The addition of 3–5 hours of supplemental light to a 10-hour photoperiod of natural light resulted in marked improvement in the quality as well as in the quantity of seeds produced. Plants grown under continuous illumination produced a relatively large number of seeds, but these were of poorer quality with respect to the percentage of heavy seeds. However, plants grown under the influence of the most favorable photoperiodic treatments produced fewer flowers during the fall and winter months than did untreated plants of comparable size grown outside in the natural light prevailing during the spring and summer.

The effect of the photoperiodic treatment was not lasting, since flower primordia were not initiated during January after the long-day treatments had been discontinued in November. Supplementing natural light with light of low intensity from an artificial source had no apparent effect on the vegetative growth under the conditions used.

In general, plants grown under ordinary greenhouse conditions without photoperiodic treatments began to produce flowers in April or early May, when the natural day length was approaching 12 hours, and continued to flower and grow vegetatively during the longer days of summer until the latter part of September, when the days were again less than 12 hours in length. During the short days of fall and winter, vegetative growth was somewhat slower, even at moderate temperatures, than during the summer months, and no appreciable number of flower primordia were ini-

TABLE 2

EFFECT OF PHOTOPERIODIC TREATMENTS ON FLOWER INITIATION AND SEED PRODUCTION BY GUAYULE

TREATMENT	HOURS OF LIGHT			WEIGHT OF SEED PRODUCED, SEPT.–DEC. (MG.)	HEAVY SEED (%)	NO. OF PLANTS THAT FLOWERED (NOV. AND DEC.)	NO. OF PLANTS THAT FLOWERED (JANUARY)	NO. OF FLOWER PRIMORDIA (JANUARY)*	NO. OF PLANTS TREATED
	Natural	Artificial	Total per day						
1	10	0	10	13	0	0	0	0	15
2	10	3	13	287	23.7	6	0	0	15
3	10	5	15	388	22.1	5	0	0	15
4	10	14	24	231	7.0	12	0	0	15
5	14.75–9.5†	0	14.75–9.5	212	16.7	0	0	0	15

* Determined by dissecting branches with aid of microscope.

† Received natural light prevailing from July 1, 1942 to Jan. 15, 1943.

tiated until the onset of vegetative growth during the following spring.

Night temperature

Methods.—Plants of variety 593, comparable with those generally used for field plantings, were obtained from a nursery in California. Fifty-four of these were selected for size and uniformity and planted in composted soil contained in 8-inch clay pots. The plants were placed on six trucks in groups of equal numbers, so that they could be transported into rooms in which specific temperatures were maintained. The number of plants in this experiment was also limited by the capacity of the equipment available.

During the summer months the plants were moved outdoors from the respective temperature-control rooms each day at approximately 6:00 A.M. and grown under the prevailing temperature and light conditions, then they were returned to controlled temperatures and kept in darkness from approximately 7:00 P.M. until the following morning, when the procedure was repeated. In October and during the following fall and winter months the same procedure was followed, except that the plants were moved each day from the respective temperatures to a greenhouse at approximately 8:00 A.M. All the plants remained at 65°–75° F. until about 6:00 P.M., when they were again returned to the respective control rooms. The plants were repotted several times during the experiment to maintain soil fertility.

Results.—The plants grew vigorously during July, August, and early September. Some plants that received 80°–90° night temperature flowered as late as February, while those exposed to lower night temperatures failed to bloom after the latter part of October. Seed collections were made as the seeds ripened during the period from August to November. The 80°–90° night-temperature treatment resulted in reduced growth, as indicated by the dry weight of the plants and the relatively few seeds, which were of poor quality with respect to the percentage of heavy ones (table 3). Plants grown with a night temperature of 65°–75° produced approximately the same number of seeds but of better quality. Plants grown at the lowest night temperature, 50°–60° F., produced more than three times as many seeds as did those grown at the two higher temperatures, and these were of relatively good quality.

TABLE 3

Effect of night temperature on vegetative growth and amount and quality of seed produced

Temperature (° F.)	Average dry weight per plant (gm.)	Total weight of seeds (gm.)	Heavy seeds (%)
50–60	37.5	16.1	25
65–75	36.1	5.1	27
80–90	23.8	5.0	10

Discussion

There is evidence that certain minerals can be mobilized within unripe fruit and seeds at the expense of the vegetative portion of the plant. Thus phosphorus was found to accumulate readily in the green fruit and seeds of tomato at the expense of phosphorus contained in the leaves, when the plants were grown on a limited supply of this element (1). Since under certain conditions developing seeds can accumulate some elements at the expense of the rest of the plant, it may be reasoned that in such instances only wide variations from the optimum nutrient supply would be

reflected in the quantity or quality of the seeds.

In the present experiments, however, guayule was found to be sensitive to the nitrogen supply, and it was not necessary to subject the plant to wide variations in the amounts available in order to influence the amount and quality of the seeds. PULTZ (6) likewise reported that the seed yield of sugar beets was greatly increased as the result of liberal applications of nitrogen, but the treatment apparently had no effect on their quality. BURTON (3) found that the application of potassium and phosphorus fertilizers to Bermuda grass resulted in increased numbers of flower heads. He also observed that nitrogen fertilization resulted in an increase in the number of heads formed on some of the grasses tested, but that seed set was not influenced by the fertilizer treatment. TOLMAN (7) found that the number of small seeds produced by sugar beets increased when nitrogen was added as a fertilizer, but this response was not obtained when both nitrogen and phosphate were added. The percentage germination of seeds was relatively high when nitrogen was added during years favorable to growth, but the addition of nitrogen during unfavorable years decreased the percentage germination.

In the present experiments also, the proportion of calcium and magnesium supplied to the plants influenced the quantity and quality of the seeds. Although the potassium, phosphorus, and sulphur was varied over wide range concentrations, these were apparently within a luxury range, which possibly accounted for the fact that they did not influence seed production under these conditions.

The initiation of flower primordia and the development of seeds were favored to some extent by exposing the plants to relatively long daily periods of illumination, and this effect appeared to be largely due to the action of the long photoperiod on some mechanism other than the process of carbon assimilation, since the response was obtained through the use of supplementary light of low intensity. On the basis of the results observed, however, guayule appears to be less sensitive to variations in the length of the daily period of illumination than are some other plants, such as certain varieties of soybean or chrysanthemum (5). It is evident that guayule is capable of producing flowers in abundance over a range of photoperiods, and that the maintenance of an optimum day length is not of vital importance in connection with seed production.

The blooming period was lengthened to some extent by exposing the plants to a relatively high night temperature, but this extension of the flowering season was not associated with a high rate of seed production, nor the production of seeds of good quality with respect to the number that developed mature embryos. These plants also made less vigorous vegetative growth. It is suggested that these responses to a high night temperature may have resulted from a high rate of respiration during periods of darkness, which would tend to deplete the plant of some materials essential for vigorous vegetative growth and seed development.

Although the direct cause of the prevalence of seeds with immature embryos is not known, these experiments indicate that the condition is more prevalent when the plant is exposed to an environment which fails to supply sufficient amounts of certain inorganic elements, particularly nitrogen, adequate photoperiod, or which does not favor the full utilization of organic materials for the

growth and development of the plant as a whole, because of relatively high night temperature.

Summary

1. In nutrient-culture experiments, the amount and quality of seeds produced by guayule was influenced by some of the nutrient elements made available to the plants. Most seeds were produced when the plants were watered with solutions containing the highest concentration of nitrogen tested, 192 p.p.m. Concentrations of sulphur were varied between 65 and 260 p.p.m., and of phosphorus between 72 and 288 p.p.m., with no apparent effect on the amount or quality of the seeds. Potassium was varied between 95 and 380 p.p.m. with no apparent effect on seed production. With respect to magnesium and calcium, the two most favorable solutions contained 78 and 117 p.p.m. magnesium and 173 and 260 p.p.m. calcium.

2. Plants were induced to bloom during the winter months by supplementing a 10-hour period of daylight with 3–14 hours of light from an artificial source. When the long-day treatment was discontinued in November, flower production decreased during December and the plants failed to bloom or initiate flower primordia during January.

3. Plants subjected to a day temperature of 65°–75° and a night temperature of 80°–90° F. initiated flowers during the winter months although the natural daily period of illumination was not extended by means of light from an artificial source. In contrast, plants exposed to the same temperatures during the day, and night temperatures of 65°–75° or 45°–55° F., failed to flower during the winter months.

4. Plants grown under conditions of relatively warm days and cool nights (45°–55° F.) produced more seeds of good quality. Plants exposed to moderate night temperatures (65°–75°) produced fewer seeds, which were also of good quality. Plants subjected to relatively high night temperatures (80°–90°) produced approximately the same number of seeds as those exposed to a moderate night temperature, but these were of relatively poor quality.

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HISTOLOGICAL STUDIES ON PARTHENOCARPIC FRUITS OF LILIUM REGALE INDUCED BY GROWTH SUBSTANCES[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 553

J. M. BEAL

Since GUSTAFSON (2) first reported the production of mature seedless fruits through the application of lanolin mixtures of indoleacetic, indolepropionic, indolebutyric, and phenylacetic acids to the styles of certain solanaceous plants, considerable additional work has been done on a variety of species. A summary of these investigations has recently been presented by the same writer (3).

The only instance in which a detailed histological study of induced parthenocarpic fruits in comparison with ones developed following pollination is that of GARDNER and KRAUS (1). They found that development of parthenocarpic fruits in holly following spraying with indoleacetic acid paralleled almost precisely that following pollination, except that cells of the stigmas of sprayed fruits proliferated more than those pollinated, did not collapse and suberize so quickly, and there was a complete lack of embryo or endosperm development in the sprayed fruits. No marked or disorderly cell proliferation occurred in the pistils to which the acid had been applied. The parthenocarpic berries averaged as large and were as turgid and green as those following pollination, and at the time of fruit ripening they showed no differences in color.

Material and methods

During the spring and early summer of 1942, ovaries on potted plants of *Lilium regale* growing in the greenhouse were treated with 1 per cent lanolin mixtures of indoleacetic, naphthaleneacetic, and naphthoxyacetic acids and with a combination consisting of equal volumes of the three 1 per cent concentrations stirred thoroughly together before using.

Before treating, the flowers were allowed to open. The stamens were then pulled off and the stigma and style removed by cutting squarely across the top of the ovary at the base of the style. The cut surface of the ovary was then covered with one of the lanolin mixtures and left for varying lengths of time. Controls were handled in the same manner except they were treated with lanolin alone or, in a few cases, were given no further treatment after removal of the style.

In none of the controls did further marked growth occur despite the occurrence of mitotic figures in carpel walls, integuments, and nucellus at the time of treatment. There was relatively slight difference in subsequent behavior of the controls, whether treated with lanolin only or given no treatment other than removal of the style. Those treated with lanolin alone dried and shriveled perhaps a little less slowly, but none enlarged to more than one and one-third their diameter at the time of treatment.

The ovaries were collected at various intervals following treatment. They were cut transversely into segments 2–4 mm. in length, fixed in Navashin's solution, and imbedded in paraffin. Sections were cut at 10 μ and stained in Flemming's triple stain.

Gross responses

Ovaries to which the three growth substances and the combination were applied all enlarged in length and diameter at approximately the same rate and attained nearly the same final dimensions

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

(fig. 1), although those treated with naphthaleneacetic acid attained slightly greater total development than the others (fig. 1*D*). The combination of the three and indoleacetic acid alone resulted in fruits of essentially the same sizes and remained green and turgid longest. The parthenocarpic fruits were firm and bright green in color for a period of 8–10 weeks, when they began to dry and shrivel. They attained as great a total length as fruits resulting from pollination but

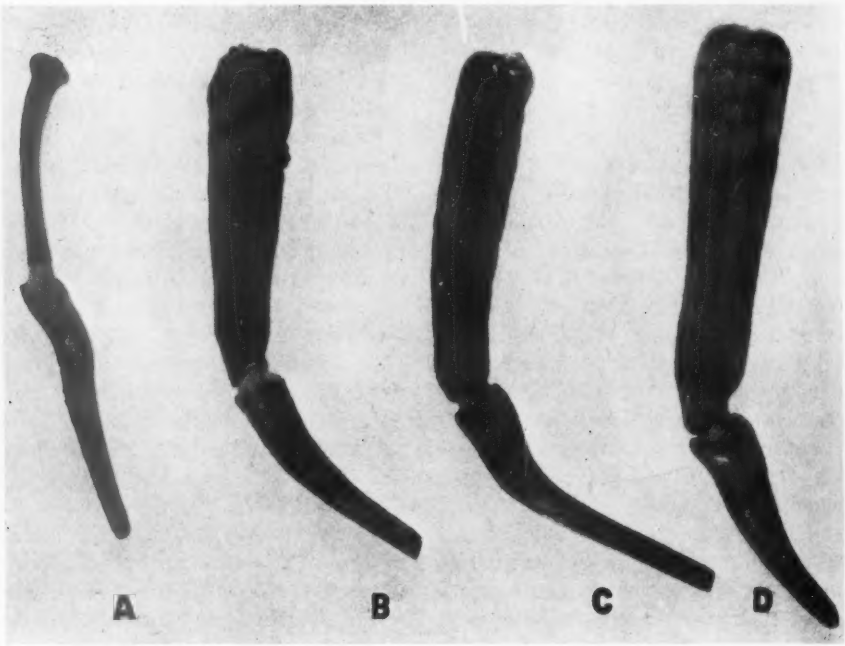

FIG. 1.—*A*, control, treated with lanolin alone, showing practically no change in size except at top end; *B*, 624 hours after treatment with combination of the three growth substances; *C*, 1152 hours after treatment with indoleacetic acid; *D*, 768 hours after treatment with naphthaleneacetic acid.

similar appearance (fig. 1*B*, *C*). A few of the treated ovaries showed initial responses to all the substances by starting to enlarge, and then growth ceased and they became dark and died. The majority of those treated responded as illustrated in figure 1*B–D*, and some at least remained green and turgid for more than 9 weeks. About fifteen of the plants were transferred to the garden in early July, transplanting from the pots being carried out without disturbing the roots. It was on these that the treated ovaries were always smaller in diameter. None of them developed embryos or endosperm.

Histological details

The photomicrographs are at two magnifications. All transverse sections of entire ovaries are at a magnification of about ×12, while those of individual ovules, both longitudinal and transverse, are magnified approximately ×60, or five times as much, in order to show more cellular detail.

The relative size and stage of development of ovaries and ovules attained at the time of treatment are shown in figure 2*A*. At this time the megagametophytes are mostly in or near the second four-nucleate stage, and mitotic figures are common in the carpel walls and in the integuments and nucellus of the ovules (fig. 2*A*, *B*). The inner integument and the nucellus have attained nearly full size, but the outer integument has not yet reached quite half the development it would show at the time of fertilization, which normally occurs about 100–110 hours following pollination in *L. regale*. The outer integument develops considerably during this period, ultimately covering the inner integument completely at the micropylar end before the pollen tube enters.

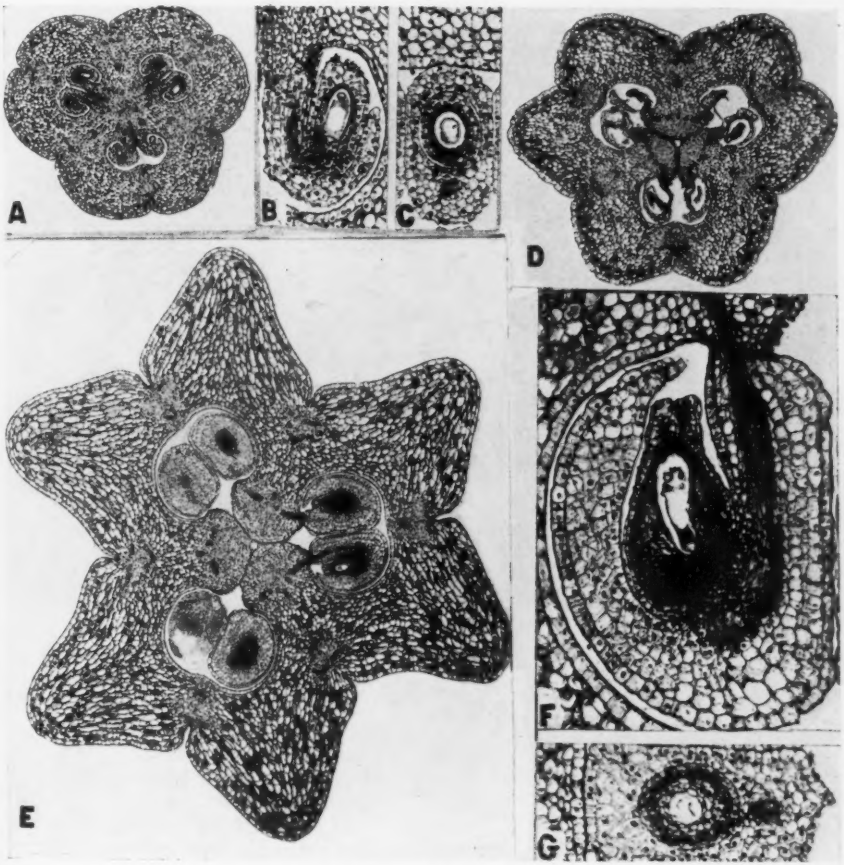

FIG. 2.—*A*, transection of control ovary at time of flower opening; *B*, ovule in longisection from same section as *A*, showing cellular detail; *C*, longisection of ovary at same stage as *A* and *B*, showing transectional view of an ovule; *D*, transection of ovary treated with lanolin alone, 552 hours after treatment; *E* transection of ovary 336 hours after treatment with naphthoxyacetic acid; *F*, ovule in longisection from *E*; *G*, transection of ovule from same ovary as *F*.

A transection of an ovary to which lanolin alone was applied, collected and fixed 552 hours after treatment (fig. 2*D*), shows evidences of enlargement in the carpel walls. Some of the growth is doubtless the result of increase in number of cells, but the greater part is a result of cell enlargement. The ovules, even though the outer integuments increased in volume, are now much shrunken and necrotic. When collected, this particular ovary was pale yellow in color and somewhat shrunken.

The ovaries treated with the growth substances, either separately or in combination, behaved in much the same way in nearly all instances. At 336 hours after treatment with naphthoxyacetic acid the carpel walls and the ovules had increased in overall size about twofold or more (fig. 2*E*). The cells of integuments were still turgid, but the nucellus showed signs of disintegration (fig. 2*F*, *G*), and the cells composing the megagametophyte were partially collapsed and shrunken.

At 480 hours after treatment with naphthoxyacetic acid (fig. 3*A*) there was little further change in the appearance of carpel walls or in the integuments (fig. 3*B*, *C*). The megagametophyte, however, had now disintegrated completely and was observable only as a line more or less at the center of the nucellus.

Ovaries treated with a combination of the three growth substances developed at about the same rate as those to which the substances were applied separately. At 624 hours following treatment with the combination (fig. 4*A*) there was greater total development in carpel walls and in

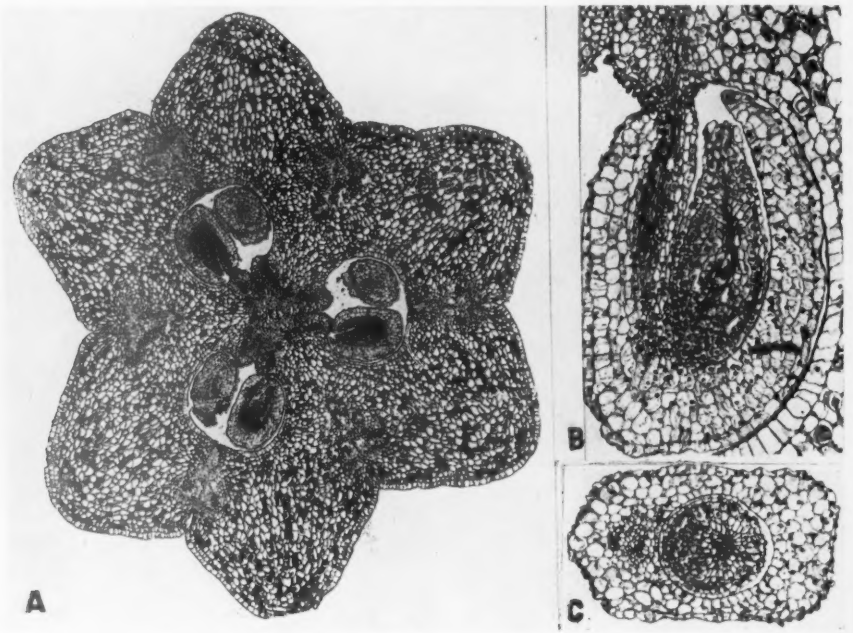

FIG. 3.—*A*, transection of ovary 480 hours after treatment with naphthoxyacetic acid; *B*, ovule in longisection from same section as *A*; *C*, transection of ovules from same ovary as fig. 3*A*.

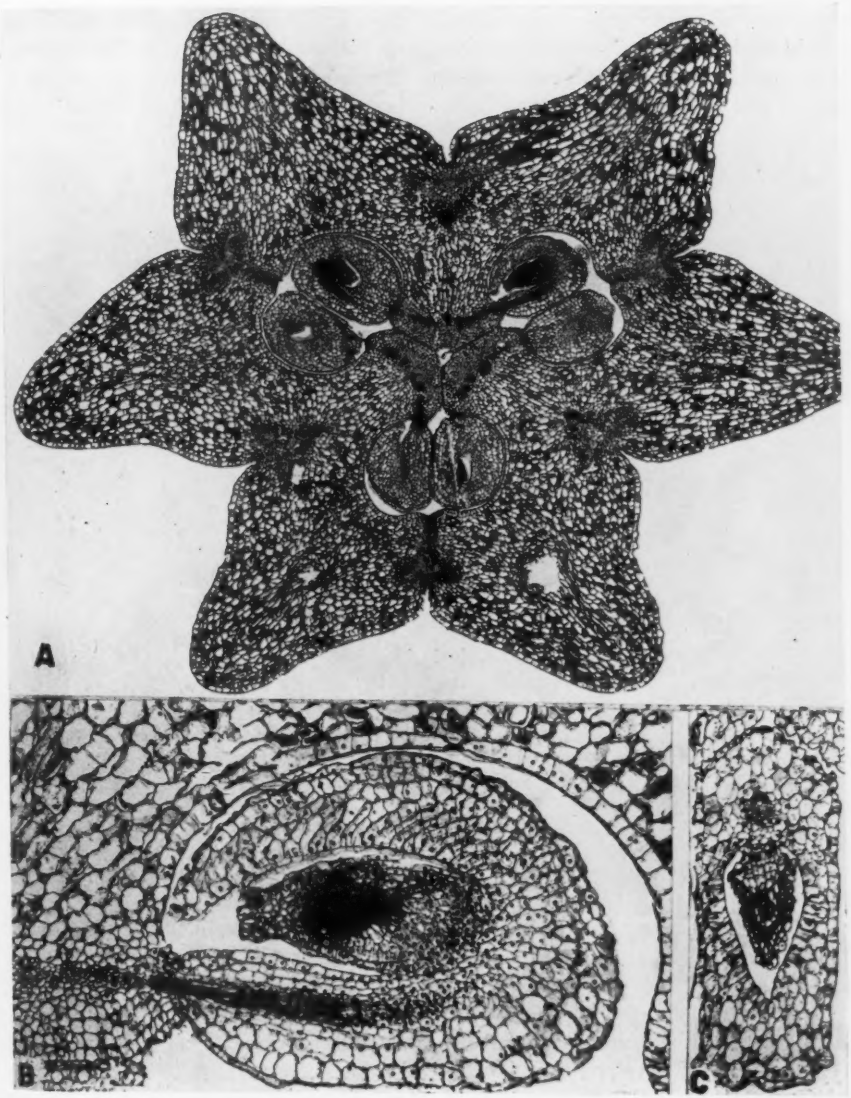

FIG. 4.—*A*, transection of ovary 624 hours after treatment with combination of the three growth substances; *B*, ovule in longisection from upper right of fig. 4*A*; *C*, transection of ovule from same ovary as fig. 4*A*.

the outer integument of ovules, but there were increasing evidences of degeneration in the inner integument and nucellus of the ovules (fig. 4*B*, *C*). Areas were also present in the carpel walls in which disintegration of cells had occurred, resulting in cavities which run parallel with the carpel walls.

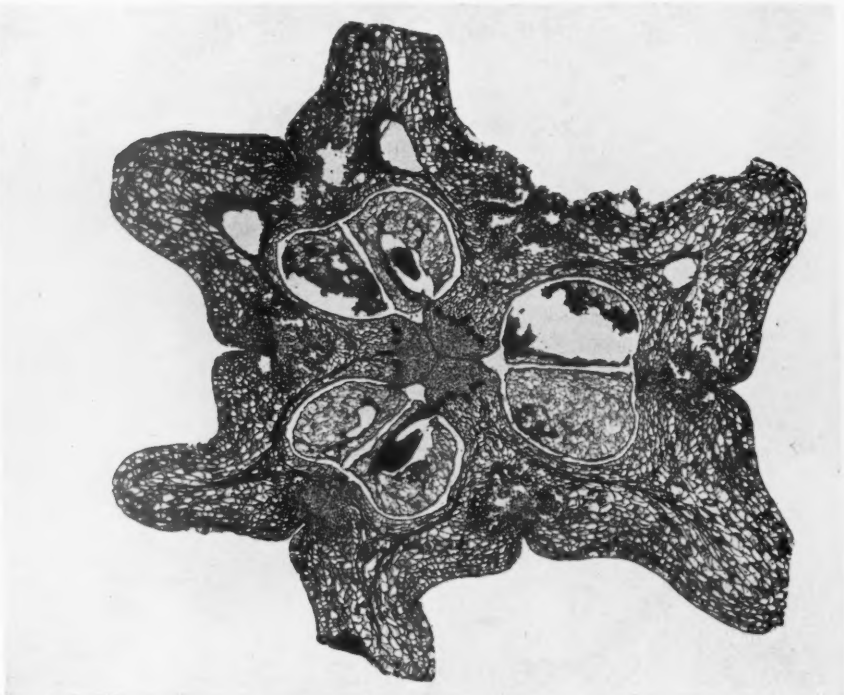

FIG. 5.—Transection of ovary 760 hours after treatment with naphthaleneacetic acid

A transection of an ovary collected 760 hours after treatment with naphthaleneacetic acid (fig. 5) gave further evidences of cellular effects in the inner portion of the carpel walls. The cavities resulting from cell disintegration were larger than those in figure 4*A*, and a band of collapsed cells was evident running almost entirely around the section. The overall size of ovules was greater, but the increase in volume was due entirely to growth of the outer integument. No cell divisions were apparent, and the increase in size was mainly the result of cell enlargement (fig. 6*A*, *B*). The inner integument and the nucellus were now undergoing disintegration.

The longest time interval following treatment at which material was collected and sectioned was 795 hours (33 days). A transection of such an ovary, treated with indoleacetic acid, showed well-developed carpel walls and large plump ovules (fig. 7). While the cavities in the carpel walls were somewhat larger than those in figure 5, there was less shrinkage and disorganization of cells in the inner walls of the carpels. But the

most striking development had taken place in the outer integument (fig. 8*A*, *B*). The marked increase in size had resulted from both cell divisions and cell enlargement, chiefly the latter. Indications of recent divisions were evident in several places in figure 8*A*, but the average volume of individual cells was much greater than in ovules at flowering time. Cells of the inner integument and nucellus were nearly all dead and much shrunken (fig. 8*B*).

The general responses shown by the ovaries of *L. regale* to the three growth-promoting substances separately and to their combination were strikingly similar. For a time they resembled closely in external appearance those which developed as a result of pollination, but approximately 400 to 500 hours after treatment no further increase in diameter took place, and they remained more slender than those in which fertilization had occurred. They were similar in color

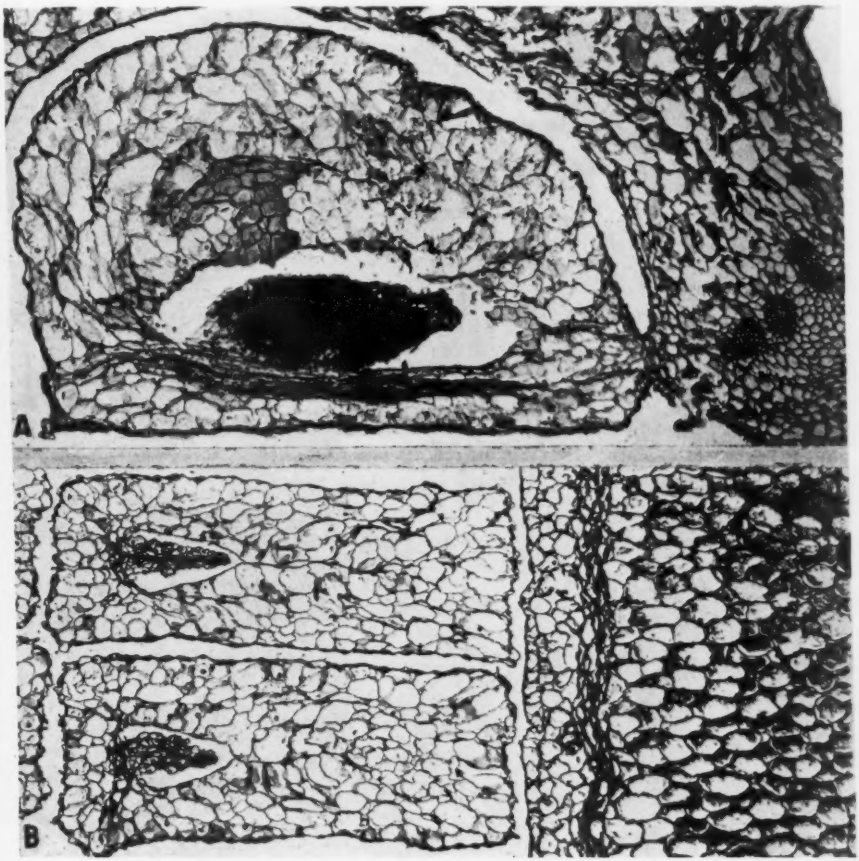

FIG. 6.—*A*, ovule in longisection from same ovary as fig. 5; *B*, transection of ovules from same ovary as fig. 5.

and felt as firm to the touch for 600 to 700 hours following treatment, after which time they usually became a paler green and began to show a slight pithiness. Both loss of color and pithiness increased slowly, until final death of the entire ovary occurred at 8–10 weeks following treatment.

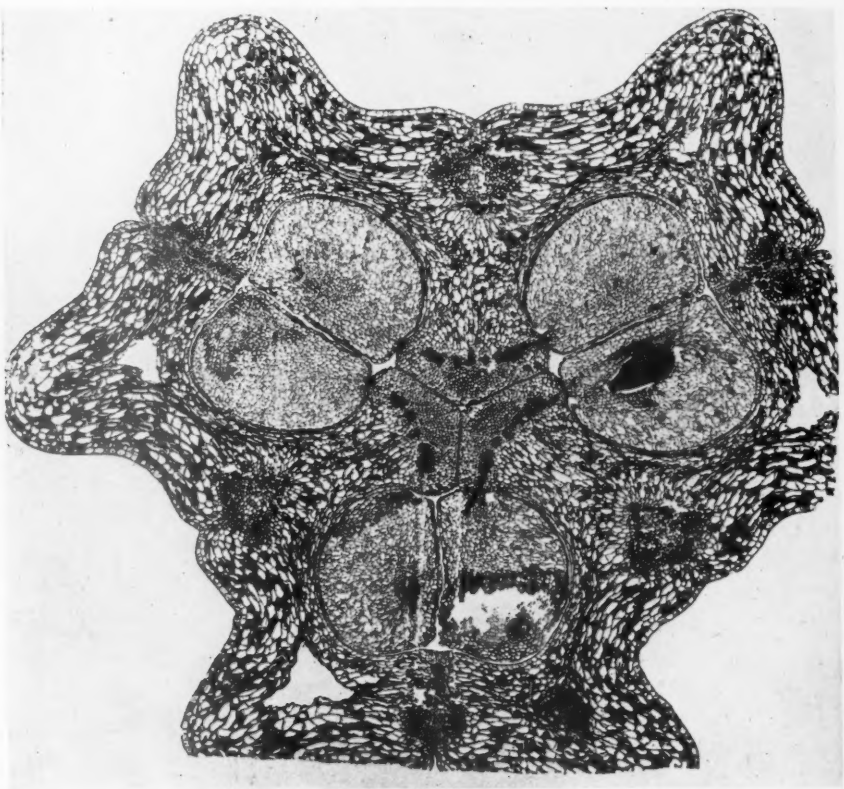

FIG. 7.—Transection of ovary 795 hours after treatment with indoleacetic acid

The development of vascular bundles in the parthenocarpic fruits and those developed following pollination showed little difference. There was noticeable difference, however, in the appearance of the inner carpel wall tissues of parthenocarpic fruits as compared with those developed following pollination. In all the parthenocarpic fruits examined there was evident collapse of cells in the inner region of the carpel walls, followed by death and dissolution of at least some cells as early as 600 hours after treatment. While this effect appeared more strongly marked in some instances (following application of naphthaleneacetic acid, fig. 5) than in others, it nevertheless occurred in all the ovaries studied after a time lapse of approximately 600 hours following application of the growth substance.

All the ovules of the parthenocarpic

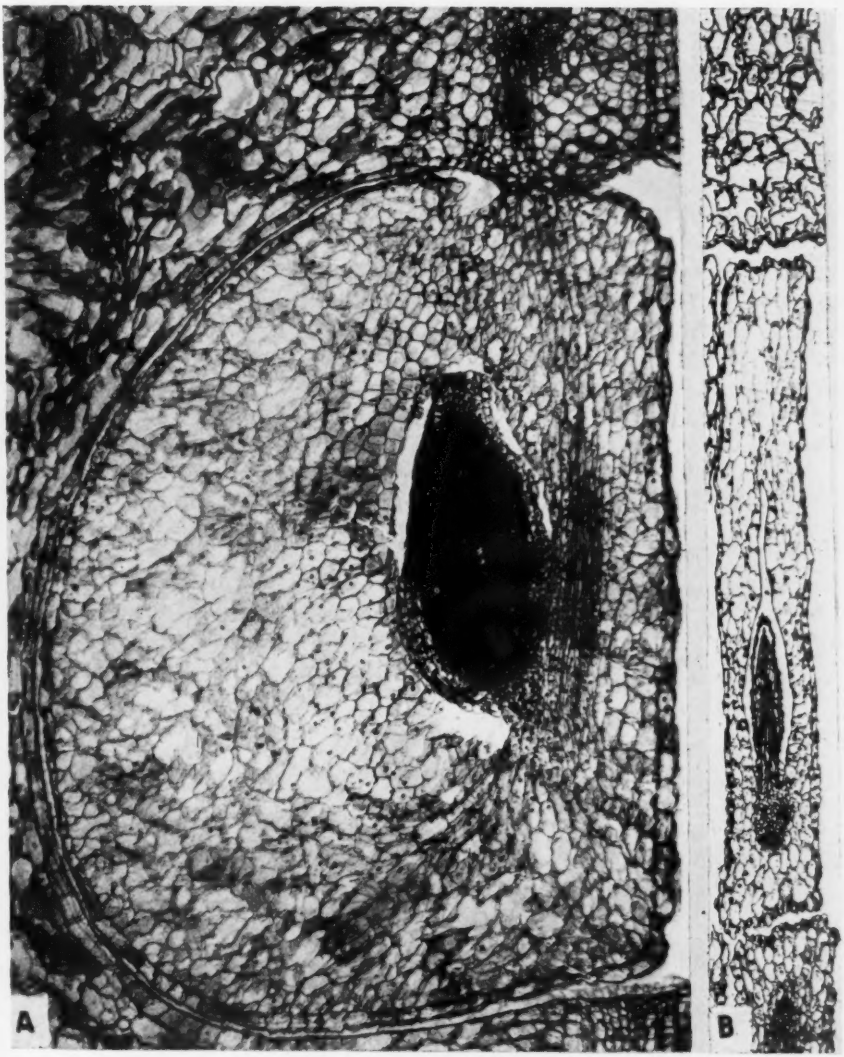

FIG. 8.—*A*, ovule in longisection from middle right of fig. 7; *B*, transection of ovule from same ovary as fig. 7.

fruits showed marked growth in the outer integument. The greater part of this growth was a result of cell enlargement rather than of cell multiplication, for once the outer integument had developed sufficiently to cover completely the micropylar end of the ovule, few additional mitoses occurred and subsequent growth was owing largely to increase in cell volume.

The most striking effects of the substances were the varied responses in specific regions and tissues of the ovaries. Few cell divisions occurred in the carpel walls subsequent to treatment. The conspicuous increase in size of ovaries resulted from enlargement of cells composing the carpels and outer integument of ovules. The shrinkage and collapse of cells in certain regions of the carpel walls, in the inner integument, the nucellus, and the mature megagametophyte occurred relatively early during the development of the parthenocarpic fruits. On the contrary, the cells of the outer integument were stimulated to increase in number to a limited extent, but chiefly they increased many times in volume. It is probable that other species of *Lilium* would show different degrees and types of responses to these same growth substances, as has been observed by WONG (4) for various species and varieties of cucurbits.

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VEGETATIVE PROPAGATION OF TARAXACUM KOK-SAGHYZ WITH THE AID OF GROWTH SUBSTANCES

P. C. MARTH[1] AND C. L. HAMNER[2]

Introduction

Seedlings of *Taraxacum kok-saghyz* grown in the United States from imported seed vary considerably, both in plant character and in rubber content of the individual plants. Published data on vegetative propagation of kok-saghyz indicate that Russian investigators have had some success with piece-root cuttings. Plants produced by this method developed quickly under field conditions.

This report is based on greenhouse experiments conducted during December, January, February, and March, 1942–1943, at the Bureau of Plant Industry Station, Beltsville, on methods of handling piece-root cuttings of kok-saghyz and their rooting response to applications of synthetic growth substances.

Material and methods

Cutting material was obtained from separate lots of plants to determine the influence of age and size of root. One lot of approximately 3000 rather large, uniform roots, 7 months old, 10–15 inches long and $\frac{3}{4}$ inches in crown diameter, was obtained from a planting at St. Paul, Minnesota. In contrast to this material, cuttings were made of relatively young plants (4 months old) grown at Parma, Idaho, having roots 6–9 inches in length and with approximately $\frac{1}{4}$–$\frac{1}{2}$-inch crowns. In addition, 3000 plants 7 months old were obtained from a planting at Klamath Falls, Oregon. These roots were long (10–15 inches), straight, with few branches, and rather comparable in size with the plants from St. Paul. Two shipments of approximately 1000 plants each were received from Berkeley and from Davis, California. The former were coarse and much branched but fairly uniform, while the latter were variable in size, owing in part to irregular germination of the seed, which took place sporadically over a long period.

Four synthetic growth substances known to induce plant responses were applied in aqueous solution and by the talc-dust method. These compounds were β-indolebutyric acid, α-naphthaleneacetic acid, α-naphthalene acetamide, and β-naphthoxyacetic acid. A mixture made up of equal portions of these four compounds was also used. Each compound and the mixture were applied to the cuttings in a range of concentrations of 10, 50, and 100 p.p.m. with the solution method, and 50, 200, and 1000 p.p.m. in the talc-dust treatment.

Experimentation

Methods of propagation

Cutting length.—In the early stages of building up a clonal population, it is desirable to use the minimum length of propagating pieces that will succeed well and produce the maximum numbers of new plants. Uniform unbranched roots, approximately $\frac{3}{4}$ inch in diameter below the crown, that were obtained from the

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St. Paul planting were made into cuttings $\frac{1}{2}$, $\frac{3}{4}$, 1, and $1\frac{1}{2}$ inches long to determine the effect of cutting length on performance. One hundred cuttings of each size were set upright in sand in a propagating case operating at 70°–75° F. Periodic examination was made of a number of cuttings of each size group at intervals of 3–4 days, and at the end of 18 days all lots were removed for examination.

It was evident during the test period that the cuttings $\frac{1}{2}$ inch long were slower in callusing and producing new leaves than longer cuttings of $\frac{3}{4}$, 1, and $1\frac{1}{2}$ inches, and at the end of 18 days 70 per cent of the former had started to decay at the base. The $\frac{3}{4}$-inch pieces appeared to have made satisfactory callus growth at both ends, but this size was somewhat difficult to form into bundles, and the cuttings (twenty per bundle) tended to slip out. The size finally selected as a standard was the 1-inch length. A small vibrating motor-type saw was found useful in securing uniform lengths (fig. 1).

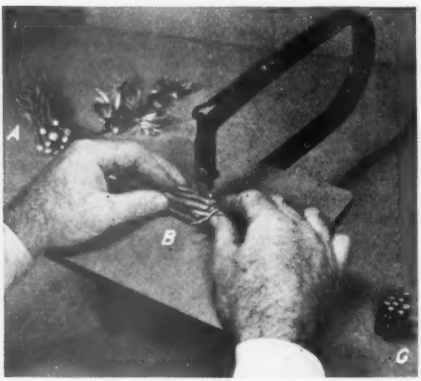

FIG. 1.—Method of making root cuttings of koksaghyz: *A*, trimmed and bunched roots with tops removed; *B*, small vibrating saw; *C*, bundle of cuttings ready to be treated.

ORIENTATION.—Orientation with regard to the morphological top is of great importance in the successful rooting of most plants. To determine whether orientation is important with roots of koksaghyz, separate lots of freshly made cuttings (St. Paul material) were set in sand vertically with the proximal end up; horizontally in a trench $\frac{1}{2}$ inch deep; horizontally on the surface of the rooting medium and covered with $\frac{3}{4}$ inch of moist sphagnum; and in an inverted position. The temperature of the cutting bench fluctuated from 70° to 80° F. during the 21-day test period. A minimum of 100 cuttings was used in each treatment, except with the inverted lot, which included 300 cuttings.

All the cuttings set with the proximal end up had developed leafy rosettes, and 43 per cent of them had developed some new root growth at the end of 21 days. In contrast with these results, out of 300 cuttings that were inverted, only four had developed leaves above the level of the media by the end of 21 days. The four cuttings that were the exception developed leafy growth on both ends. The remainder of the inverted lot (296) had all developed etiolated leaves below the surface and were very poorly rooted.

The humidity and temperature of the greenhouse or other imposed conditions apparently were very favorable for development of root rot, since all the cuttings laid horizontally and covered with $\frac{1}{4}$ inch of sand had decayed by the end of 21 days. In contrast, the cuttings placed horizontally on the surface of the sand and covered with moist sphagnum were in excellent condition at the end of the same period. Shoot and root growth, however, was much less extensive than with similar cuttings placed in sand in a vertical position with the proximal end up.

DEPTH OF SETTING.—Top growth was delayed and the incidence of decay was

greater with cuttings covered by the sand or soil rooting media. Three lots (100 in each) of 1-inch cuttings from the roots from St. Paul were set vertically in sand in a propagating case (70°–80° F.) at three depths of insertion: (*a*) top of cutting $\frac{1}{4}$ inch above media level; (*b*) top at media level; and (*c*) top $\frac{1}{4}$ inch below media level. By the end of 10 days all cuttings posed cuttings developed greater callus than did those that were buried. At this temperature, however, cuttings buried under 1 inch of sand remained in good condition without loss from rot organisms over a 30-day storage period (March 16 to April 15).

CUTTING BENCH AND ROOTING MEDIA.—Under the greenhouse conditions em-

FIG. 2.—Plants from root cuttings placed in sandy soil in greenhouse on February 20, 1943; removed and photographed June 23. *A*, treated with 50 p.p.m. indolebutyric acid (16 hours); *B*, untreated controls.

in the two lots with tops above or at media level had developed top callus upon which green leaves were visible. Both of these lots were maintained in the cutting bench without loss over a period of 18 days. On the other hand, the cuttings set $\frac{1}{4}$ inch below media level were just starting to callus at the end of 10 days, and 20 per cent had started to decay at the base at the end of 18 days. Likewise, in a separate experiment with cuttings held in sand in controlled temperature storage (40° ± 1° F.), the ex-

ployed in this study, inclosed cases were not necessary. Satisfactory rooting was obtained with open-type benches in a greenhouse at 60°–65° F.

Both a light sandy soil and a fine grade of washed bank sand were used as rooting media. Fifty cuttings of the Parma, Idaho, material were set in the sand on February 20 and left undisturbed for 30 days, while a similar lot was set in the sandy soil. By the end of the 30-day period the leaves on the cuttings in sand had begun to yellow, and approximately

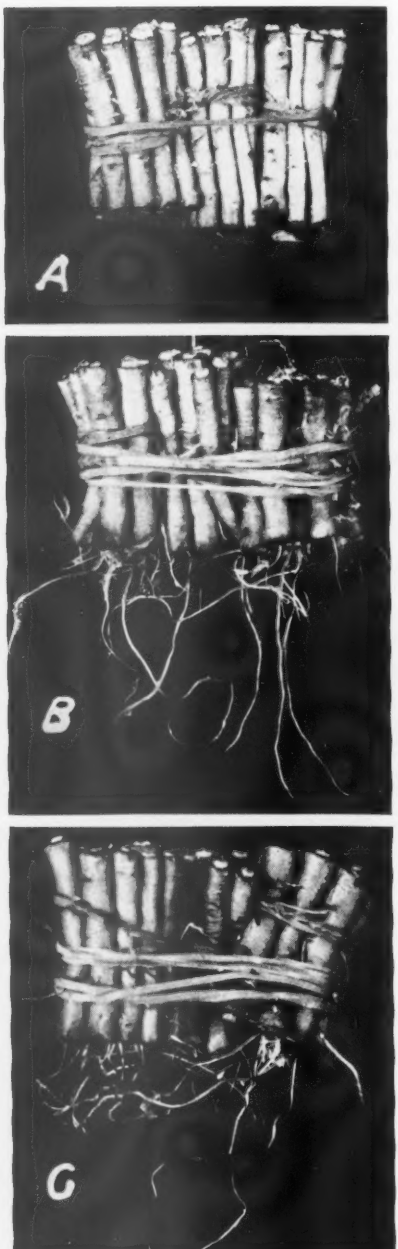

FIG. 3.—Root cuttings held in storage at 40° F.: *A*, control; *B*, solution-treated with 100 p.p.m. indolebutyric acid (16 hours); *C*, with 50 p.p.m.

60 per cent had made sufficient new root growth so that they survived potting into composted soil. Of the original fifty cuttings set in soil on February 20, forty-eight were living on June 23. A number of the resulting plants of this lot are shown in figure 2*B* as well as a comparable lot (fig. 2*A*) treated with growth substance (indolebutyric acid, 50 p.p.m.).

The wide variation in size found among these plants, the largest of which weighed 23.6 gm. while the smallest weighed but 1.8 gm. (fresh weight), was apparently due to differences in diameter size of the original cutting and to its relative position in the root from which it was made.

TEMPERATURE OF ROOTING MEDIA.—It was evident early in the work that rooting was affected by variation in temperature. Propagating cases with thermostatically controlled bottom heat were therefore used. The temperature ranges selected for trial were 60°–65°, 70°–75°, and 80°–85° F. Fifty cuttings (Klamath Falls, Oregon, plants) which received 50 p.p.m. indolebutyric acid (16-hour soaking) and fifty untreated cuttings were set in sand media maintained at each of the three temperatures on January 5.

Observations on these cuttings at periodic intervals indicated that the rate of callus production was noticeably faster at the higher temperature (80°–85° F.). At the end of 10 days all the cuttings at this temperature, both treated and untreated, had produced callus and green leaves over the entire exposed upper ends. Likewise, most of the cuttings in the 70°–75° frame were noticeably green at the upper cut surface. The cuttings set at 60°–65°, in contrast, were still almost completely dormant with but a very narrow ring of callus present on a few of them.

Final counts, when all lots were removed after 21 days in the cutting bench,

indicate that early callusing and shoot production are not reliable evidence of good rooting performance. The cuttings at the lower temperature had rooted in a much higher percentage, both in treated and untreated lots, and there was also no loss from decay. The percentage of cuttings with new roots and of dead cuttings at each of the three temperatures was as follows: Treated at 60°–65° F.: 76 per cent rooted, 0 dead; control 42 per cent rooted, 0 dead. Treated at 70°–75° F.: 74 per cent rooted, 22 per cent dead; control 32 per cent rooted, 16 per cent dead. Treated at 80°–85° F.: 8 per cent rooted, 86 per cent dead; control 18 per cent rooted, 72 per cent dead.

Treatment with 50 p.p.m. indolebutyric acid was apparently injurious at the higher temperatures (80°–85°), both in rooting and loss of cutting, while the same treatment applied to cuttings rooted at 60°–65° caused a highly significant increase in rooting without loss. Further evidence that growth substances are effective in inducing root production at even lower temperature was found in comparable treated (50 p.p.m. indolebutyric acid) and untreated cuttings stored in moist sphagnum and held in a controlled-temperature storage room at 40° F. for 30 days. During the storage period no new roots developed on the untreated cuttings, whereas 85 per cent of the treated ones had developed new roots, many of which were 3–4 inches in length (fig. 3).

Effectiveness of growth substances on rooting of cuttings

The use of growth substances as an aid in propagation of horticultural plants by stem cuttings is now common practice (**2**). Lindner (**1**) obtained a stimulating effect on rooting of horse-radish root pieces. Stuart and Marth (**3**), however, working with scion roots of twenty-six apple varieties, found no beneficial effects from use of growth substances.

TABLE 1

Effectiveness of growth substances on rooting performance and survival of plants from kok-saghyz root cuttings*

Compound†	Concentration (p.p.m.)		Percentage	
	Sixteen-hour soaking treatment	Talc-dust treatment	Rooted cuttings after 18 days (Dec. 29–Jan. 16)	Original cuttings which developed into plants, June 30
Ibc	100	.....	97	27
	50	.....	100	87
	10	.....	70	70
	...	1000	60	60
	...	200	50	50
	...	50	47	43
Nac	100	.....	100	10
	50	.....	100	77
	10	.....	63	47
	...	1000	60	53
	...	200	60	57
	...	50	53	43
Nad	100	.....	87	53
	50	.....	90	70
	10	.....	73	60
	...	1000	53	53
	...	200	47	40
	...	50	47	43
Nxy	100	.....	57	20
	50	.....	73	73
	10	.....	60	60
	...	1000	67	53
	...	200	57	57
	...	50	63	60
Mixture	100	.....	93	47
	50	.....	93	67
	10	.....	67	53
	...	1000	60	57
	...	200	63	60
	...	50	43	43
Controls	16-hours in water	.....	40	40
	.....	Talc	37	33
	Untreated	.....	30	27

* Thirty cuttings per treatment.

† Ibc, indolebutyric acid; Nac, naphthaleneacetic acid; Nad, naphthalene acetamide; Nxy, β-naphthoxyacetic acid; mixture, equal parts of the four growth substances.

The data in table 1 were obtained with cuttings of unbranched roots grown at Parma, Idaho. The thirty 1-inch cuttings included in each treatment were selected at random, so that each lot consisted of an equal number (ten) from the upper (proximal), middle, and lower portion of the plants. The upper cuttings were approximately $\frac{1}{2}$–$\frac{5}{8}$ inch in diameter, while the lower cuttings were not less than $\frac{3}{16}$ inch. The cuttings were set in sand (68°–75° F.) on December 29, 1942, and removed after 18 days (January 16), at which time record was made of the rooted cuttings in each lot. These cuttings were then potted in soil and held in a cool (50°–65° F.) greenhouse. By April 26 a majority of the resulting plants had developed leaf rosettes 3–4 inches long, at which time they were field-planted. Counts were again made on June 30 of the living plants in each rooting treatment (table 1).

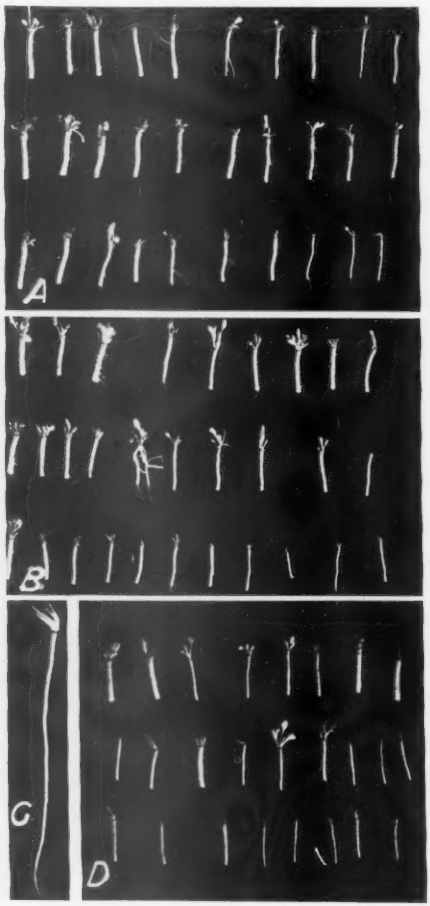

FIG. 4.—Root cuttings not receiving growth-substance treatment: *A*, water control (soaked 16 hours); *B*, untreated control (set directly into rooting medium); *C*, typical plant from which cuttings were made; *D*, talc dust only, control. Cuttings set in sand December 29, 1942; photographed 18 days later.

Comparison of the effectiveness on rooting of the individual growth substances and the mixture employed can best be made with the 16-hour soaking treatments, rather than with the talc-dust method. As shown in table 1, a marked increase over the controls was obtained from each growth substance applied in talc at the highest concentration used, 1000 p.p.m., and a large percentage of the rooted cuttings subsequently developed into plants. However, 100 per cent rooting was not approached in any of the dust treatments, nor was there any evidence of injury. It is possible, therefore, that the optimum concentration of growth substances in talc was not used in the particular range selected.

Although increased rooting over the control lots (fig. 4) was obtained from each of the growth substances and the mixture when applied in solution, the response to each compound was somewhat different.

INDOLEBUTYRIC ACID.—Following treatment with this growth substance, the plants tended to produce long fine roots, many of which arose from the new callus at the base of the cutting. Judging from observations on cuttings stored in

moist sphagnum, this compound induced more rapid rooting than the other growth substances. At a concentration of 50 p.p.m. (soaking treatment), 100 per cent rooting was obtained, and 87 per cent of these cuttings subsequently developed (table 1). At the stronger concentration of 100 p.p.m. the cuttings were injured (fig. 5). This is reflected in the very low number of plants (27 per cent) derived, even though the actual rooting percentage (97 per cent) was high at this concentration.

NAPHTHALENEACETIC ACID.—This compound proved a very potent root-stimulating agent, inducing many short thickened lateral roots on the treated cuttings (fig. 6). It also was much more toxic at the higher concentration (100 p.p.m.) than the other compounds, and although all cuttings in this treatment had rooted strongly, 63 per cent showed injury when removed from the rooting media. The injury is further indicated by the low number of plants (10 per cent) resulting. The optimum concentration ap-

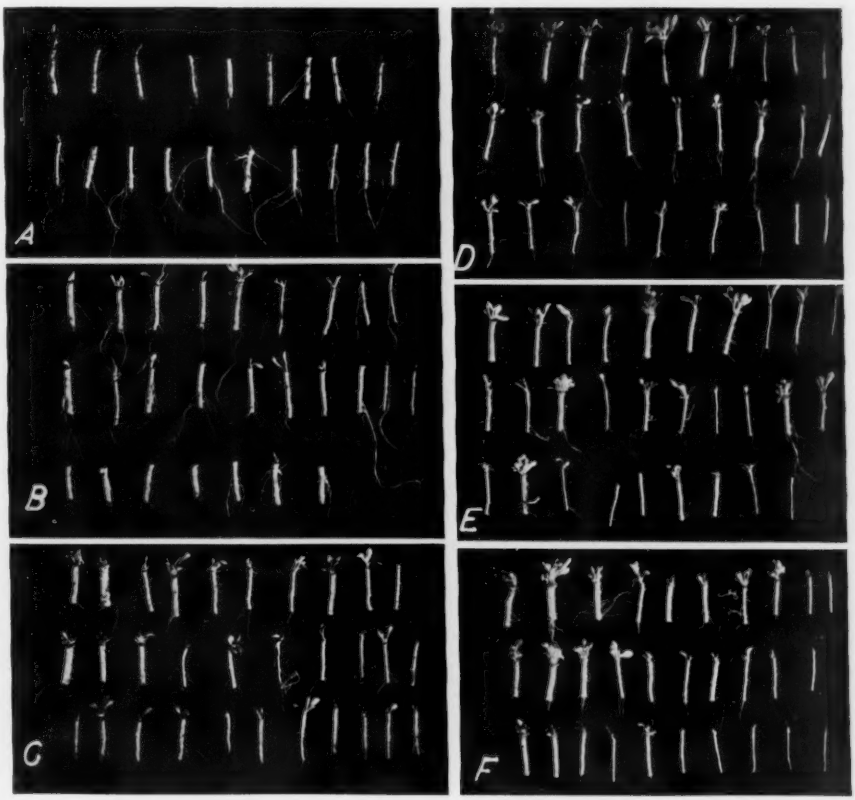

FIG. 5.—Root cuttings treated with indolebutyric acid: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

pears to be around 50 p.p.m. with the 16-hour soaking treatment. As indicated in table 1, all cuttings in this treatment were rooted after 18 days, and 77 per cent produced plants.

mum concentration suitable for this type of material was approached. As shown in table 1, 50 p.p.m. with this compound gave the maximum rooting and ultimate plant yield. The roots developed on

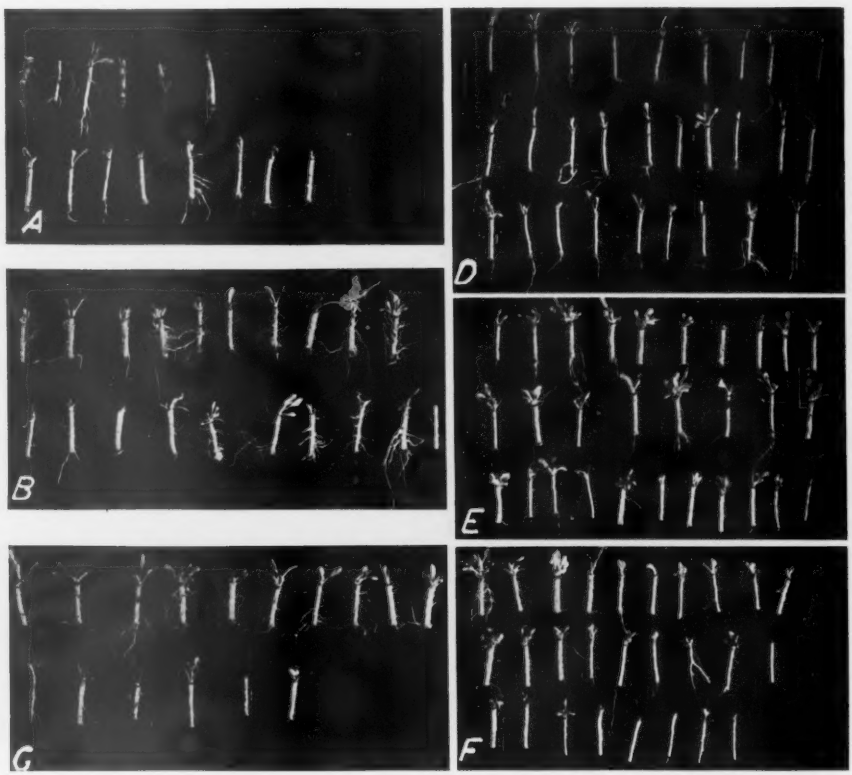

FIG. 6.—Root cuttings treated with naphthaleneacetic acid: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

NAPHTHALENE ACETAMIDE.—This compound caused a marked increase in root production in comparison with the controls at each of the concentrations used—100, 50, and 10 p.p.m. (fig. 7). The increase, however, was less than with indolebutyric or naphthaleneacetic acid. Since some injury was caused at 100 p.p.m., it is assumed that the maximum concentration suitable for this type of material was approached. As shown in table 1, 50 p.p.m. with this compound gave the maximum rooting and ultimate plant yield. The roots developed on treated cuttings were thickened but slightly longer than those resulting from naphthaleneacetic-acid treatment.

β-NAPHTHOXYACETIC ACID.—Following treatment with this compound, cuttings had few, but very long fine roots (fig. 8). On the basis of root production alone, β-naphthoxyacetic acid would be rated much less satisfactory than the

other three compounds. However, with the exception of the 100 p.p.m. treatment, where injury occurred, the sparsely rooted cuttings resulting from treatment yielded fairly high percentages of plants (table 1).

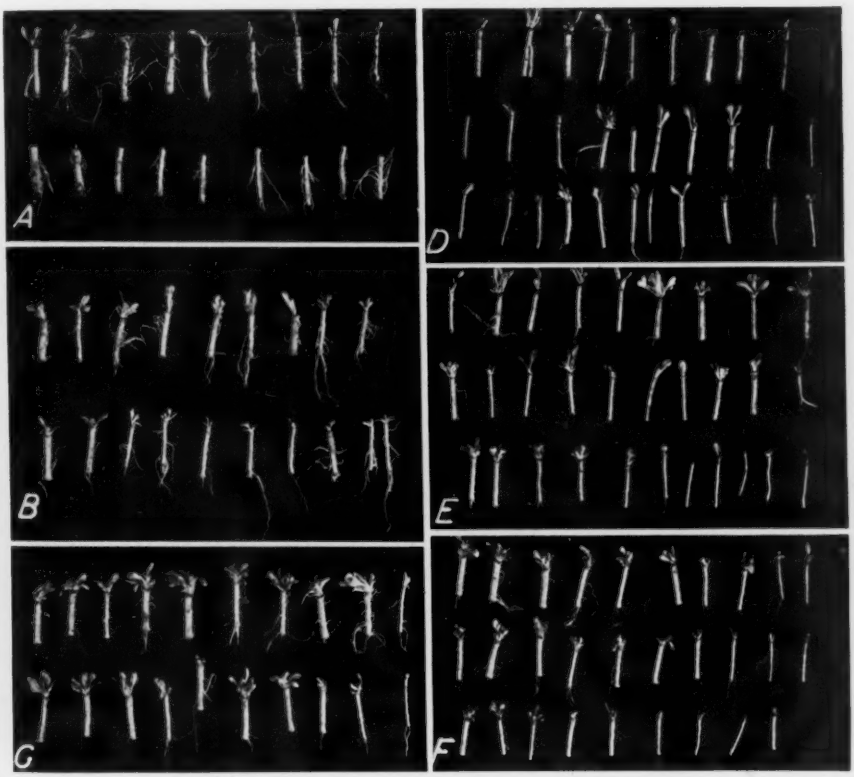

FIG. 7.—Root cuttings treated with naphthalene acetamide: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

MIXTURE.—The maximum rooting obtained with the use of a growth-substance mixture (at 50 p.p.m.) was not so great as with either indolebutyric or naphthaleneacetic acid alone. At 100 p.p.m., however, the mixture was noticeably less toxic than any of the compounds applied singly (fig. 9). Perhaps in its favor also is the fact that the type of roots developed on the cuttings treated with the mixture tended to be somewhat intermediate between the long fine roots characteristic of the cuttings treated with indolebutyric acid and the short coarse roots of those treated with naphthaleneacetic acid.

GROWTH-SUBSTANCE TREATMENT OF INTACT PLANTS

After the roots have been cut into small (1-inch) pieces, there is some difficulty in telling which is the morphologi-

cal top. It is relatively easy for the bundles to become reversed in the course of handling and many are likely to be planted upside down. There would be some advantage, therefore, in having a satisfactory growth-substance treatment for the intact plants before the cuttings were made. The cuttings could then be made up from the treated roots just prior to insertion in the rooting medium, with less likelihood of reversing them.

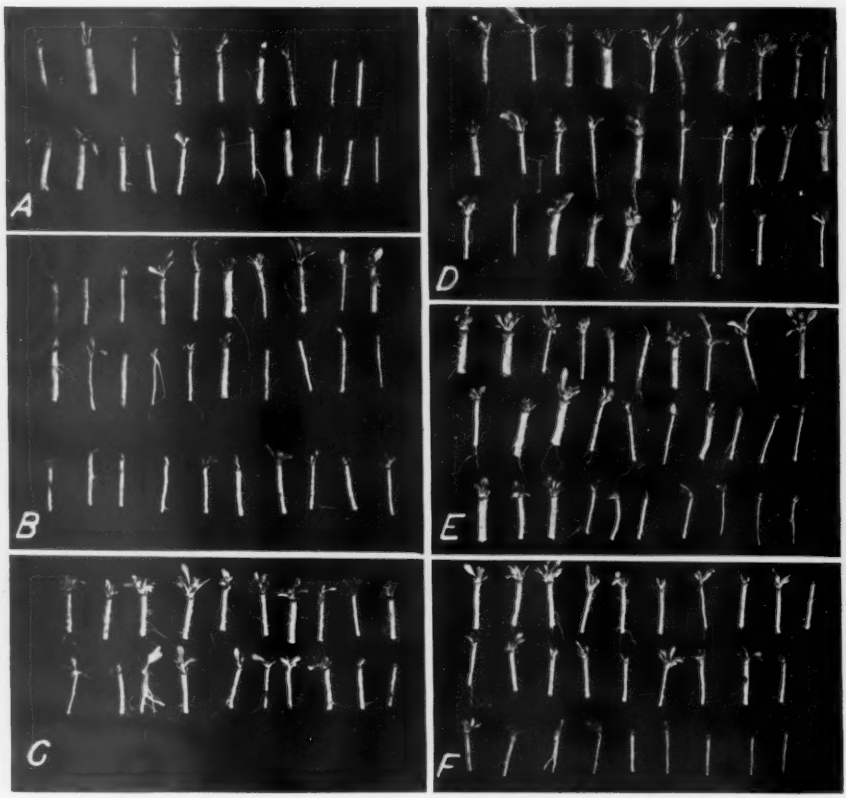

FIG. 8.—Root cuttings treated with β-naphthoxyacetic acid: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cutting set in sand December 29, 1942; photographed 18 days later.

Uniform lots of ten entire plants grown at Klamath Falls, Oregon, were soaked for 16, 32, and 64 hours in aqueous solutions of 0, 10, 50, and 100 p.p.m. of each of the four growth substances and the mixture, while similar lots were soaked for 2, 4, and 8 hours in stronger solutions of 0, 200, 400, 800, 1000, and 1400 p.p.m. of the same compounds. For comparison, an additional control lot was planted without soaking. The attempt was thus made to compare a treatment consisting of a long soaking period at dilute concentrations with a shorter soaking period at higher concentrations. Upon removal

from the solutions, the plants of each treatment were cut into uniform pieces 1 inch long, thereby yielding 75–80 cuttings in each lot. The rooting medium was a sandy loam soil, and the last treated lot was set on February 2, 1943. Final record on all was obtained at the end of 30 days (March 4).

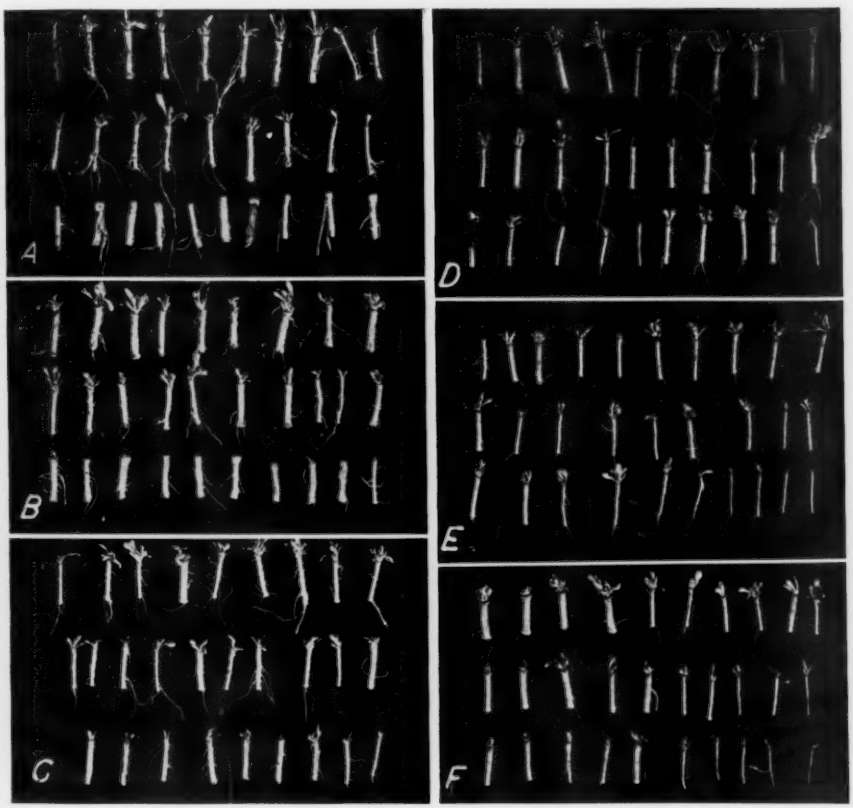

FIG. 9.—Root cuttings treated with mixture of growth substances: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

Under the conditions employed, increased rooting did not result from treatment of intact plants with growth substances before the cuttings were made. Although the results were complicated by considerable damage in all lots from rot organisms, greater loss of cuttings was found in the treated than in the untreated lots, with the exception of the 16-hour treatments at 10 and 50 p.p.m. and of the 2- and 4-hour treatments at 200 and 400 p.p.m. The percentage of rooted cuttings in each of these lots treated with growth substance was no greater than that from either the untreated plants or those soaked in water only.

Prolonged soaking for 32 and 64 hours, either in water only or with growth sub-

stances added, appeared to be harmful. Likewise strong concentrations of 1000 and 1400 p.p.m. with 8 hours of treatment also appeared definitely injurious.

greenhouse, potted in soil, and held in a cool (40°–55° F.) greenhouse and then planted in the field on April 20, produced an abundance of flowers. Many of the

FIG. 10.—Upper: plants developed from root cuttings. Lower: close-up of some of same plants to show the profuse flower development obtained.

All the cuttings in each of these lots died as a result of rot or other causes by the end of the 30-day period.

FIELD DEVELOPMENT OF PLANTS FROM CUTTINGS.—The majority of the plants from cuttings that were rooted in the

larger plants had rosettes 5–10 inches in diameter and had produced 25–40 flowers per plant by June 20 (fig. 10). During warm days, however, all the plants from cuttings were severely wilted by noon but returned to a turgid condition in the

evening. A number of plants were therefore lifted for root examination.

In contrast to the relatively large amount of top growth produced by these plants, root growth was much restricted. Even the larger (10-inch) ones had developed but two to three sizeable roots per plant, the longest of which was but 2 inches.

Apparently the top-root ratio of kok-saghyz plants produced from root cuttings may be markedly altered by cultural conditions imposed upon the developing plants (fig. 2). Root cuttings set in soil in a cool greenhouse (60°–65° F.) on February 20 had developed much greater root than top growth by June 23.

Summary

1. Root cuttings of more than 9000 plants of *Taraxacum kok-saghyz* were used in experiments during December, January, February, and March, 1942–1943, to determine the response of cuttings to variations in propagation methods and to applications of β-indolebutyric, naphthaleneacetic, and naphthoxyacetic acid, and napthalene acetamide. Each compound was used in concentrations of 10, 50, and 100 p.p.m. in solution treatments and at 50, 200, and 1000 p.p.m. in talc dust.

2. The most desirable length of cutting was 1 inch, with a minimum diameter of $\frac{3}{8}$ inch. Cuttings $\frac{1}{2}$ inch in length were slow in callusing and rooting, while cuttings $\frac{3}{4}$ inch long were somewhat difficult to handle and form into bundles.

3. The roots had strong polarity, and most pronounced rooting responses were obtained with cuttings set with the proximal portion up. Inverted cuttings failed to develop normally, while those laid horizontally were retarded in growth of tops and roots.

4. Under greenhouse conditions, cuttings with tops at or slightly above the level of the rooting medium developed new top and root growth more quickly than cuttings buried $\frac{1}{4}$ inch below the surface.

5. Both sand and a light sandy soil were used as a rooting medium with equal success. The cuttings set in soil developed into sizeable plants over a 4-month period, while with similar lots set in sand it was necessary to remove them to soil at the end of 30 days.

6. Although root cuttings developed callus and top growth more quickly at 80°–85° F., these cuttings were more susceptible to rot and the percentage rooted was lower than with similar cuttings maintained at 60°–65° during the rooting period (21 days).

7. Treatment with growth substances by the solution methods resulted in an increase in the percentage of rooted cuttings in comparison with untreated controls. The optimum concentration for best results with the compounds used was at 50 p.p.m. with a 16-hour solution treatment. Each of the four growth substances and the mixture resulted in injury at 100 p.p.m.

8. The growth-substance–talc-dust treatments induced a higher percentage of rooted cuttings than was found in the untreated lots. At the highest concentration (1000 p.p.m.), however, rooting was less than with the same compounds applied at 50 p.p.m. in solution.

9. At the optimum concentration of growth substance for rooting (50 p.p.m.) indolebutyric and naphthaleneacetic acids produced a higher percentage of rooting than either naphthalene acetamide, β-naphthoxyacetic acid, or the mixture used.

10. The roots produced by cuttings

following treatment with indolebutyric and β-naphthoxyacetic acids tended to be long and fine, while those which developed with napthaleneacetic acid and naphthalene acetamide tended to be short and much thickened.

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EFFECTS OF HIGH TEMPERATURE ON METAPHASE PAIRING IN LILIUM LONGIFLORUM

S. L. EMSWELLER AND PHILIP BRIERLEY[1]

The regularity of nuclear and cell divisions in plants has been considerably affected by many agents, such as X-rays, ultraviolet light, various chemicals, diseases, and abnormal temperatures. Both low and high temperatures, as well as a sudden change from one to the other, have been shown to be particularly effective.

The writers have previously briefly discussed (4) some of the meiotic irregularities that arose in *Lilium longiflorum* as the result of a sudden change from low to high temperature. In the present paper these abnormalities are presented in some detail.

Two horticultural forms of *L. longiflorum* were employed. One was the Creole variety that is grown in Louisiana and along the Gulf Coast and the other was the Japanese type known as Giganteum. The latter should not be confused with the species *L. giganteum* Wallich. The Creole variety is believed to be a clone because of its uniformity, its self-incompatibility (2), and certain cytological peculiarities not observed in any of the many other *longiflorum* varieties so far examined (5). The variety Giganteum is actually a mixture of various types, as is shown by its variability in many flower characteristics, its stem color, and the incompatibility relations between individual plants.

Twelve Creole and nine Giganteum plants, all with buds in which some of the anthers were at the leptotene and early pachytene stages, were selected. At this time the buds on the Creoles ranged 13–32 mm. in length, and those on the Giganteums 8–36 mm. One week before the treatments were given, the temperature in the greenhouse where the plants had been growing was lowered to 10°–13° C. at night and 13°–16° C. during the day. On March 19 the plants were divided into two lots. Six Creoles and five Giganteums were moved to a warmer greenhouse, where the temperatures were 13°–16° C. at night and 16°–19° C. during the day. The remaining six Creoles and four Giganteums were placed in a chamber where the temperature was raised in about 3 minutes to 45°–46° C. A thermocouple placed inside one of the buds showed that the temperature was maintained at 45.5° ± .5° C. during the entire exposure of 30 minutes. High humidity was maintained by introducing steam into the chamber. Following the treatment the plants were placed in the warm greenhouse with the untreated ones. Anthers were collected at intervals from both treated and untreated plants, starting when many of the pollen mother cells were in first metaphase. The time required for such development varied to some extent, apparently being dependent on the size of the bud at the time it was exposed to the high temperature treatment. The anthers were fixed in 3:1 absolute alcohol-acetic acid, then transferred through several stages of alcohol to 80 per cent, where they were stored until slides were made. The ordinary

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aceto-carmine smear technique was used, and the photomicrographs were made with a Zeiss microscope and Miflex camera.

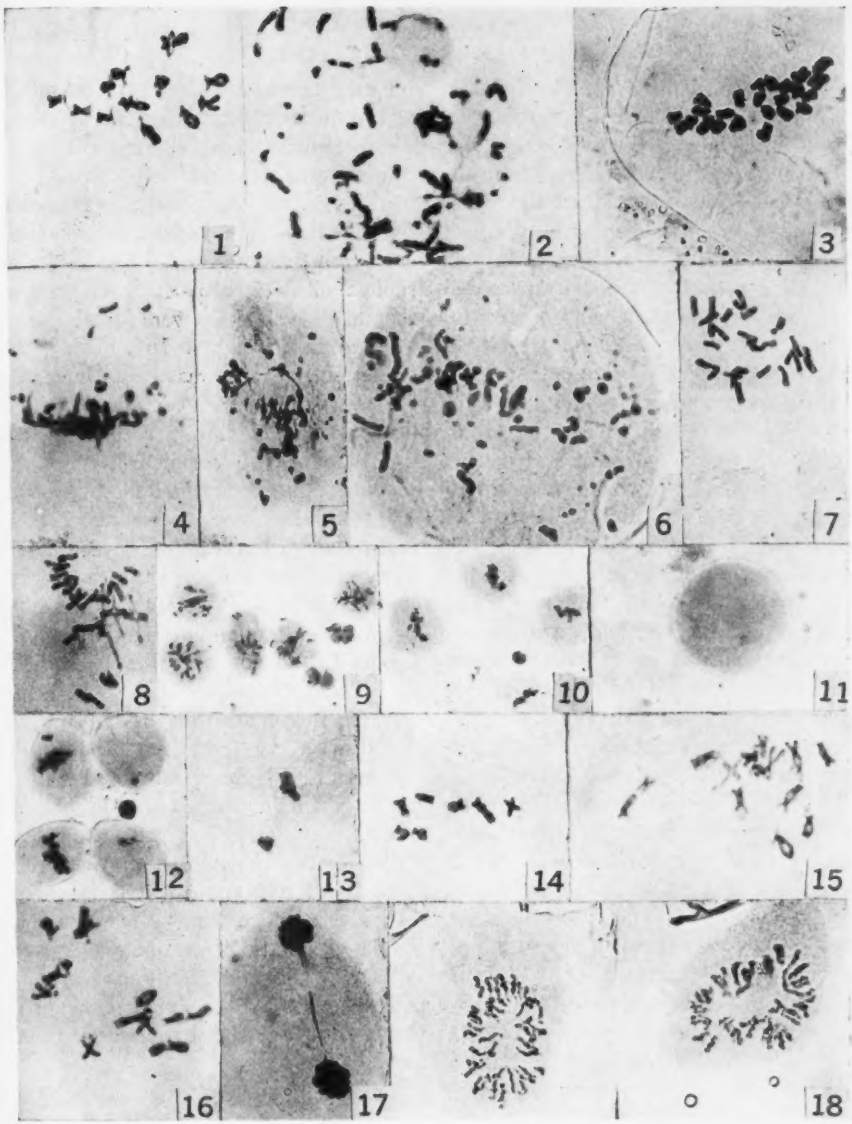
Some remarks regarding metaphase bivalent formation in the untreated Creole and Giganteum plants have already been presented (5). The more detailed data on chromosome pairing in the untreated plants are presented in table 1. It was possible to examine carefully only one flower each from three of the Creoles and two each from the remaining three plants. As shown in the table, univalents were found in eight of the nine buds investigated. In addition there was an unusual condition in which the chromosomes were broken into small fragments, in many instances so numerous that it was impossible to count them. The condition is referred to in this paper as "blown-up." Four cells of this type are shown in figures 2, 4, 5, 6. Figure 2 was collected from an untreated Creole plant on March 23, 91.5 hours after the others were treated; figures 4 and 6 are from treated plants collected March 25, 138.5 hours after treatment; figure 5, also treated, was collected March 27, 48 hours later. Figure 1 is a normal Creole pollen mother cell with 12 bivalents, shown for comparison with the blown-up cells. It was collected at the same time as figure 2. Some of the blown-up cells (figs. 4, 6) closely resemble irradiated ones, while others (fig. 5) are very similar to the condition found in sticky gene of maize (1).

TABLE 1

METAPHASE PAIRING IN TWO VARIETIES OF *LILIUM LONGIFLORUM* (UNTREATED)

Plant no.	Flower bud no.	No. cells observed	Cells showing chromosomal conditions indicated				
			$12_{II}+0_I$	$11_{II}+2_I$	$10_{II}+4_I$	Blown-up cells	Large p.m.c.
			Creole				
1	1	74	51	19	4	.....	.....
2	1	49	40	9	.....	.....	.....
3	1	16	13	2	1	.....	.....
	2	142	93	16	.....	33	.....
4	1	137	116	8	.....	13	.....
	2	71	68	.....	.....	1	2
5	1	132	100	26	.....	4	2
6	1	46	36	7	.....	3	.....
	2	67	56	7	.....	3	.....
Total	.....	734	573	94	5	57	4
			Giganteum				
1	1	49	49	.....	.....	.....	.....
	2	50	50	.....	.....	.....	.....
	3	33	32	1	.....	.....	.....
2	1	40	40	.....	.....	.....	.....
3	1	48	48	.....	.....	.....	.....
4	1	59	57	2	.....	.....	.....
5	1	68	68	.....	.....	.....	.....
Total	.....	347	344	3	.....	.....	.....

This unusual fragmentation in blown-up cells is first seen at late prophase. The long chromosomes appear to be broken into many pieces, most of which have a tendency to be attached to the general mass by fine attenuated threads. These cells are always larger than the surrounding normal ones and are found scattered throughout the anther. At earlier stages of meiosis large cells have also been observed, but they do not show any indication of the fragmentation seen in the pollen mother cells at later satges. At first metaphase the blown-up cells usually show an apparent spindle, and occasionally a few abnormal bivalents may be seen among the mass of fragments. Some of the large fragments apparently move to both poles, since second metaphases have been observed in which some of the many chromosomes appeared to be undergoing normal mitotic splitting. Occasional microspores are seen that contain only a few chromosomes, and it is possible that some of these arose from blown-up cells At all stages of development these abnormal cells are larger than usual, as may be seen by comparing figures 1 and 2, photographed at the same magnification.



FIGS. 1–18.—*Lilium longiflorum:* Fig. 1, pollen mother cell of Creole variety with 12 bivalents. Fig. 2, "blown-up" pollen mother cell from untreated Creole plant. Fig. 3, large Creole pollen mother cell with 24 shortened chromosomes, resulting from suppression of heterotypic division (from same bud as fig. 2). Figs. 4–6, blown-up cells from treated Creoles. Fig. 7, pollen mother cell from bud 1 of treated Creole plant 3, showing complete asynapsis. Fig. 8, another pollen mother cell from same bud as fig. 7, showing 3 bivalents and 18 univalents. Fig. 9, five pollen mother cells from one anther of bud 1 of Creole plant 3, showing variable asynapsis. Fig. 10, three pollen mother cells from bud 2 of same plant as fig. 9, showing complete asynapsis. Fig. 11, enucleate pollen mother cell from bud 2 of Giganteum plant 1. Fig. 12, four pollen mother cells from same source as fig. 11, one with 12 bivalents, one with 11 plus 2 univalents, one with a heteromorphic bivalent, and one with a fragment. Figs. 13–16, pollen mother cells from same Giganteum plant as figs. 11 and 12, with 1 bivalent plus a fragment, 7, 14, and 13 bivalents, respectively. Fig. 17, chromatid bridge in pollen mother cell of treated Creole plant. Fig. 18, second metaphase in Creole, probably later stage of fig. 3. Figs. 1–4, 6, 13–17, ×600; figs. 5, 11, ×400; figs. 7, 8, 18, ×500; figs. 9, 10, 12, ×300.

The blown-up cells are closely associated with ones showing normal behavior, and their frequency varies from plant to plant and even in buds from the same plant. This variation (table 1) has been observed each year during the last four growing seasons. Sometimes anthers have been collected and slides prepared that did not contain a single blown-up cell; a week later slides made from a younger bud on the same plant have shown this condition. In the anthers of untreated Creoles there also occurs an occasional large pollen mother cell with 24 shortened chromosomes. One is shown in figure 3. This was from the same plant and collection that produced the blown-up cell shown in figure 2. Only four cells of this type were observed among the 734 recorded in table 1. They are always larger than normal, being about the same size as the blown-up cells. All four observed in the untreated plants were at the same stage of development as the one shown in figure 3. Such cells probably divide to form diploid dyads. Figure 18 shows the two nuclei of such a dyad, each with 24 chromosomes.

The first metaphase in Giganteum is very regular (table 1). Three buds on plant 1 and one each on the remaining four plants were examined. Only three in the 347 cells observed showed any divergence from complete pairing, and in these only 2 univalents per cell were found. Additional observations of meiosis in Giganteum during other seasons have never revealed any of the blown-up or large pollen mother cells found in the Creole variety.

The effects of the 30-minute exposure at 45°–46° C. on the Creole and Giganteum plants were not so severe as might have been expected. Some buds showed external injury, and a few were aborted. No buds collected for observations on meiosis showed any indication of injury as a result of the treatment. The effects on metaphase pairing are recorded in tables 2 and 3. In the Creole three buds were examined from plant 1, two each from plants 2, 3, and 5, and only one bud each from plants 4 and 6. As shown in table 2, all the irregularities occurring in the untreated Creoles were found in each of the treated plants. In addition, the degree of asynapsis was greatly increased in flower-bud number 1 of plant 3 and in bud number 2 of plant 5. A cell from bud 1 of plant 3 with complete asynapsis is shown in figure 7. Figure 8 shows another cell from the same source, with 3 bivalents and 18 univalents. Every possibility of chromosome association, from complete pairing to complete asynapsis, was found, although not in any one flower bud. In addition, a few first metaphase figures with fragments were seen. Some of these fragments were probably associated with the formation of dicentric chromatids. Figure 17 shows a cell from a treated Creole plant with a chromatid bridge that has broken at two points. Unfortunately the available anthers were rather limited in number, and not many late stages of meiosis were observed. Similar chromatid bridges have been seen in untreated Creole plants; no data are available as to whether the frequency is increased by the high temperature treatment.

Treatment on two buds on the same plant shows divergent effects. In figure 9 is shown a group of five highly asynaptic cells from Creole bud number 1 of plant 3 for comparison with the regular pairing (fig. 10) in the three cells from bud 2 of the same plant. There is also considerable difference in frequency of large pollen mother cells and blown-up cells in the two buds of plant 3 (table 2). The blown-up cells in bud 2 were found in groups of

TABLE 2

TABLE 2

METAPHASE PAIRING IN TWO VARIETIES OF LILIUM LONGIFLORUM (HIGH TEMPERATURE TREATMENT)

PLANT NO.	FLOWER BUD NO.	NO. CELLS OBSERVED	CELLS SHOWING CHROMOSOMAL CONDITIONS INDICATED														
			$12_{II}+0_I$	$11_{II}+2_I$	$10_{II}+4_I$	$9_{II}+6_I$	$8_{II}+8_I$	$7_{II}+10_I$	$6_{II}+12_I$	$5_{II}+14_I$	$4_{II}+16_I$	$3_{II}+18_I$	$2_{II}+20_I$	$1_{II}+22_I$	$0_{II}+24_I$	Blown-up cells	Large p.m.c.
			Creole														
1.....	1	117	29	29	6	2	0	0	0	0	0	0	0	0	0	45	6
	2	17	11	4	0	0	0	0	0	0	0	0	0	0	0	2	0
	3	77	10	10 (1+f)	3	0	5	0	0	1	0	0	0	0	0	45	2
2.....	1	88	54	4	3	0	0	0	0	0	0	0	0	0	0	26	1
	2	56	9 (1+f)	20	9	5 (1+f)	0	0	0	1	0	0	0	0	0	12	0
3.....	1	59	0	0	0	1	0	0	0	6 (1+f)	8	9	13	9	10	3	0
	2	201	42	22	5	1	1	0	0	1	0	0	0	0	0	102	27
4.....	1	71	52	9	0	0	0	0	0	0	0	0	0	0	0	10	0
5.....	1	264	210	2	0	0	0	0	0	0	0	0	0	0	0	46	6
	2	62	0	3 (1+f) (1+3f)	1	1	2	5	6	0	0	0	0	5	4	35	0
6.....	1	170	58	33	5	0	1	0	0	0	0	0	0	0	3	56	14
Total.....		1182	475	136	32	10	9	5	6	9	8	9	13	14	17	383	56
			Giganteum														
1.....	1	82	0	0	0	0	0	0	0	0	0	4	8	15	55	0	0
	2	(see table 3)															
2.....	1	39	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	36	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	39	37	2	0	0	0	0	0	0	0	0	0	0	0	0	0
.....	1	52	0	0	1	1	3	4	4	12	13	8	3	3	0	0	0
.....	1	(see table 3)															
Total.....		248	112	2	1	1	3	4	4	12	13	12	11	18	55	0	0

three to five, but it is difficult to be certain of such relationships in a smear preparation.

The data in table 2 on the treated Giganteums show almost complete inhibition of chromosome pairing in flower-bud number 1 of plant 1 and greatly reduced pairing in the bud from plant 3. Plant 2 formation was very high. The two buds were probably at different stages of development when treated, with bud number 2 probably in a pre-meiotic condition. From bud number 2, 192 cells were examined critically and found to fall into thirty-nine types with regard to their chromosome content and behavior.

TABLE 3

METAPHASE PAIRING IN LILIUM LONGIFLORUM, VARIETY GIGANTEUM (HEAT TREATED)

CONDITION OF PAIRING	NO. OF CELLS SHOWING CONDITION INDICATED		CONDITION OF PAIRING	NO. OF CELLS SHOWING CONDITION INDICATED	
	Flower bud no. 2, plant 1	Flower bud no. 1, plant 4		Flower bud no. 2, plant 1	Flower bud no. 1, plant 4
Enucleate	4	1	II+1	2	1
f	2		II+2	2	
1+o	10	1	II+3+ff	1	
2+o(1+f)	7		II+f	2	
2+clumps	2		12+o	36	43
3+o	6		12+1	2	
4+o	5		12+1+f	1	
4+2 clumps	1		12+2	2	1
$4+1_{III}$	1		12+f	1	
5+o	5	1	13+o	36	17
5+2 clumps	1		13+1	2	
6+o	5		13+2	2	
6+clump	1		13+1+f	1	
7+o	5		13+f	1	1
7+clump+f	1		13+ff	1	
8+o	5		14+o	19	
8 senescent clumps	1		14+f	1	1
9+o	6	1	15+o	2	
9+6	1				
10+o	2	1	Total	192	70
11+o	7	1			

exhibited no effect in any of the three buds examined. No large pollen mother or blown-up cells were found in any of the treated Giganteum plants. The observations on bud 2 of plant 1 and on the one of plant 4 are presented in table 3. The differences in types of abnormalities (tables 2 and 3) between the two buds on the Giganteum number 1 are striking. All the cells from the first bud were diploid and showed a high frequency of asynapsis. The cells from the second bud were mostly aneuploids, and bivalent These types included enucleate pollen mother cells and those with every number of bivalents, from 1 to 15. Some of these types also had one or more fragments; and one with 4 bivalents included a trivalent. The occurrences of types most frequently found were: thirty-six cells with 12 bivalents (2n), thirty-six with 13 bivalents (2n + 2), nineteen with 14 bivalents (2n + 4), and ten with 1 bivalent (2n − 22).

The pollen mother cells in the bud from plant 4 (table 3) did not exhibit

such a wide assortment of types as did those in the bud of plant 1. There was a much greater frequency of the normal type, with 12 bivalents, but there was also a high frequency of cells with 13 bivalents (2n + 2). Since there always existed the possibility of some chromosomes being lost because of the smear technique, the data are from only those cells about which there was no question regarding this point. Some of the cells from bud 2 of plant 1 and the one bud from plant 4 are shown in figures 11–16. In figures 11 and 12 the cells were not flattened, and the unbroken cell walls can be seen. Figure 11 is typical of the enucleate cells, and figure 12 shows a group of four cells, all different with respect to the quantity or behavior of their chromatin content. The topmost cell of the four has 11 bivalents and 2 univalents; the one below and to the right has only a small fragment; the cell below and to the left has 12 bivalents; and the lowermost cell has a heteromorphic bivalent. Figures 13 and 14 show two deficient types, the former with 1 bivalent and a fragment and the latter with 7 bivalents. Types with 14 and 13 bivalents, respectively, are shown in figures 15 and 16.

Discussion

The Creole and Giganteum varieties of *L. longiflorum* reacted very differently to the same heat treatment, although as a rule these varieties are highly cross-compatible (2). In general, the meiotic irregularities observed in both varieties exposed to 45°–46° C. for 30 minutes may be divided into three classes: (*a*) those concerned with rearrangements within individual chromosomes; (*b*) those in which partial to complete asynapsis was induced; and (*c*) those in which either the entire nucleus was eliminated or chromosomes were added to or subtracted from the normally expected number.

The first group includes the so-called blown-up cells and the fragments in other cells. The origin of the blown-up cells is obscure. Although pollen mother cells in an anther are closely associated in the same environment, only a few blown-up cells were formed. Nevertheless, since the high temperature did materially increase the frequency of blown-up cells, the aberration may to some extent be controlled by the environment. All these blown-up cells were larger than normal, the increase being already present in prophase cells, so that the breakdown must have occurred during the very early stages of meiosis or possibly during the last pre-meiotic mitosis. This entire problem needs further investigation. The same aberration has been found by STEWART (9) to occur naturally in pollen mother cells of *L. pumilum*. The affected cells in this species are also larger than normal and show the same high frequency of fragmentation as occurs in *L. longiflorum*. They also occur with very high frequency, whereas only a few were found in *L. longiflorum*. The *L. pumilum* plants in which the cells occurred were from a group of seedlings many of which did not reveal any blown-up cells. It will be interesting to see whether this peculiar behavior is found in other members of the genus *Lilium*.

The fragments found in cells not blown-up probably arise from crossing-over in inverted sectors as well as from non-homologous associations. Chromatid bridges and fragments were seen in cells from both treated and untreated plants.

The suppression of chromosome pairing was greatly increased by the heat treatment. In complete asynapsis the

chromosomes lay scattered in the cytoplasm. Such cells were comparable in size with those in which pairing was complete (figs. 1, 7). The large pollen mother cells with 24 shortened chromosomes, however, apparently had a different origin. These large cells were always found in anthers in which the other cells were almost all at first metaphase. They probably resulted from suppression of the first division, although it is difficult to understand how their volume was increased so rapidly.

The complete elimination of all chromatin, or the addition or subtraction of chromosomes, in two different heat-treated Giganteum plants probably occurred at the last pre-meiotic mitosis. Enucleate cells, and ones with but a fragment, can persist up to the first meiotic division. In some cells with but 5–6 bivalents the chromosomes were about to separate, indicating that such cells can at least initiate meiosis. Unfortunately later stages of meiosis were not available, since all anthers were collected as soon as first metaphase figures were found.

The effect of exposure to high temperature on nuclear and cell divisions in other species of plants has received considerable attention from other investigators. TAKAGI (10) found that a 3–3.5-hour exposure of *Lychnis sieboldii* to a temperature of 38°–39° C. produced three types of asynapsis. In the first type the heterotypic division was suppressed, the unpaired chromosomes split, and diploid dyads were formed. The second type differed from the first in that the 24 unpaired chromosomes were assorted at random, forming tetrads of two types with respect to chromosome number. In the third type each univalent split in prophase of the first division and no interkinetic nuclei were formed, the chromosomes lying scattered in the cell. SAX (7) subjected plants of *Rhoeo discolor* that had been grown for 2–3 days at 10° C. to a temperature of 36° C. for 1 day. This resulted in suppression of meiosis and the production of dyads which developed into diploid pollen grains. Some tetraploid pollen grains were also formed as a result of the failure of nuclear division following chromosome splitting. Later the same investigator (8) studied the effect of sudden temperature changes on meiosis in *Tradescantia paludosa*. The treatments were most effective when plants were transferred from 8° to 38° C. Such chromosomal aberrations as interchanges, inversions, fusions, and fragmentations were induced. Asynapsis, or suppression of chromosome segregation at meiosis, was also produced, resulting in diploid pollen grains. DERMEN (3) found that fragmentation, fusion, and chromatid bridges were induced in *Rhoeo discolor* by changing from either a low to a high temperature or the reverse. Tetraploid pollen mother cells were formed as a result of doubling the number of chromosomes in pre-meiotic tissue of the anthers. KAGAWA (6) studied the effect of abnormal temperature on the course of pollen formation in a genus hybrid, *Triticum compactum* × *Secale cereale*. The plants were first grown at 4° C. for 39 hours, then at 30° for 7 hours. The results were classed into: (*a*) nonreduction (asynapsis); (*b*) formation of restitution nuclei; (*c*) formation of a nucleus containing all the chromosomes of the pollen mother cell in one of the two cells formed by the first division, while in the other cell no chromatic content was observed. The third abnormality sometimes varied because of the failure of all chromosomes to be included in one of the two cells formed by the first division. As a result, one of the cells

would contain most of the chromosomes and the other only one or two. This is the same type of behavior that occurred in two of the Giganteum plants.

Summary

1. Plants of the Creole and Giganteum varieties of *Lilium longiflorum* with young flower buds were removed from a cool greenhouse (10°–16° C.) and exposed for 30 minutes to a temperature of 45°–46° C. They were then returned to a greenhouse with temperature of 13°–19° C.

2. As a result of this treatment, the frequency of all the irregularities normally found in Creole plants was greatly increased. These included (*a*) so-called "blown-up" cells, in which all or practically all the chromosomes were broken into numerous fragments; (*b*) formation of restitution nuclei; and (*c*) increase in the degree of asynapsis from partial to complete. In Giganteum, partial to complete asynapsis was induced, and in two buds on different plants enucleate pollen mother cells were found as well as others with only a fragment or with bivalents ranging in number from 1 to 15. The treatment evidently affected both the last pre-meiotic mitosis and the early stages of the meiotic cycle.

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CELL GROWTH AND NUCLEIC ACIDS IN THE POLLEN OF RHOEO DISCOLOR

THEOPHILUS S. PAINTER

Introduction

The studies of CASPERSSON and coworkers, BRACHET, and others have shown that when there is rapid synthesis of complex substances such as proteins taking place in cells, as in the formation of ova, embryonic development, malignant or other types of growth, or the elaboration of complex secretory products (pancreas), considerable quantities of ribose nucleic acids are invariably present in the cytoplasm and usually heavy deposits of chromatin in the nuclei.[1] The presence of ribonucleic acid in the cytoplasm causes the latter to stain with basic dyes, and in older publications, especially on plant cytology, many have indicated the presence of basophilic materials in growing tissues. Not all basophily in the cytoplasm is due to the presence of ribonucleic acids, however, and the identification of the latter can be made either through ultraviolet absorption studies (**5**) or by the use of ribonuclease enzymes linked with appropriate methods of preservation and staining (**2**). The latter method has been employed in the present study.

In *Rhoeo* there are three periods of marked increase in the size of pollen mother cells and their derivatives by true growth (that is, the synthesis of new cytoplasmic and nuclear materials). The first of these includes the usual growth which the pollen mother cells undergo just prior to the meiotic divisions and represents about a fourfold increase in cell volume. The second period of growth comes as the microspores change into mature pollen grains and represents about a fifteenfold volume increase. The third period begins when pollen is placed on the stigma of the flower and includes the formation of the pollen tube.

Material and methods

Incidental to another experimental study of chromosomes, it was noted that in *Rhoeo* the cytoplasm becomes extremely basophilic in microspores as they undergo development into mature pollen. Tests showed that this basophily is removed when the microspores are first treated with ribonuclease, indicating that the basophilic material is made up of ribose nucleic acids.[2] For the study reported here, BRACHET's technique (**2, 3**) was employed. Anthers in various stages of development were dissected from flower buds and preserved in Helly's fluid. Sections were either stained in Unna's methyl green-pyronin mixture or were treated first with ribonuclease (kindly furnished by Dr. Kunitz) in the following manner. Sections were brought into distilled water in which a small amount of crystalline ribonuclease was dissolved. Such slides were kept at a temperature of about 50° C. for 2–3 hours. Control slides were similarly treated except for the ab-

[1] The term ribose nucleic acid is used throughout this paper in the sense of polymerized ribose nucleotides and thus is not restricted to the four nucleotides carrying adenine, cytosine, guanine, and uracil, which are conventionally represented in most diagrams of ribose nucleic acid.

[2] See ALLEN, F. W., The biochemistry of the nucleic acids, purines, and pyrimidines. Ann. Rev Biochem. 10:221. 1941.

sence of the enzyme. After treatment and washing in distilled water, experimental and control slides were placed back to back, stained, differentiated, and dehydrated together, so that each pair of slides received just the same treatment. Anthers dissected in aceto-carmine give about the same color reactions found by the longer Helly-Unna procedure. A number of anthers were also fixed in Navashin's fluid, stained by Feulgen's method, and counterstained in light green.

In following the changes in the anthers during the development of pollen, and those which occur when pollen is placed on the stigma of the flower, interest centers primarily on the reaction of the cytoplasm of the pollen mother and other cells to the basic dye pyronin. When basophilic material is present it causes the cytoplasm to stain a bright red with pyronin, and since the basophilic material is removed by ribonuclease the intensity of the red color in the cytoplasm of untreated cells is taken as an indication of the amount of ribonucleic acid present at a given stage. In the drawings the various shades of red in the cytoplasm are represented by corresponding shades of gray and black, and very great care has been exercised in trying to represent comparable degrees of color intensity in the same way in all drawings. After Helly's fixation the red color seems localized in coarse fibrous strands, or sometimes in granules, but after aceto-carmine or Navashin's fluid the basophilic material is not associated with any particular cytoplasmic constituent.

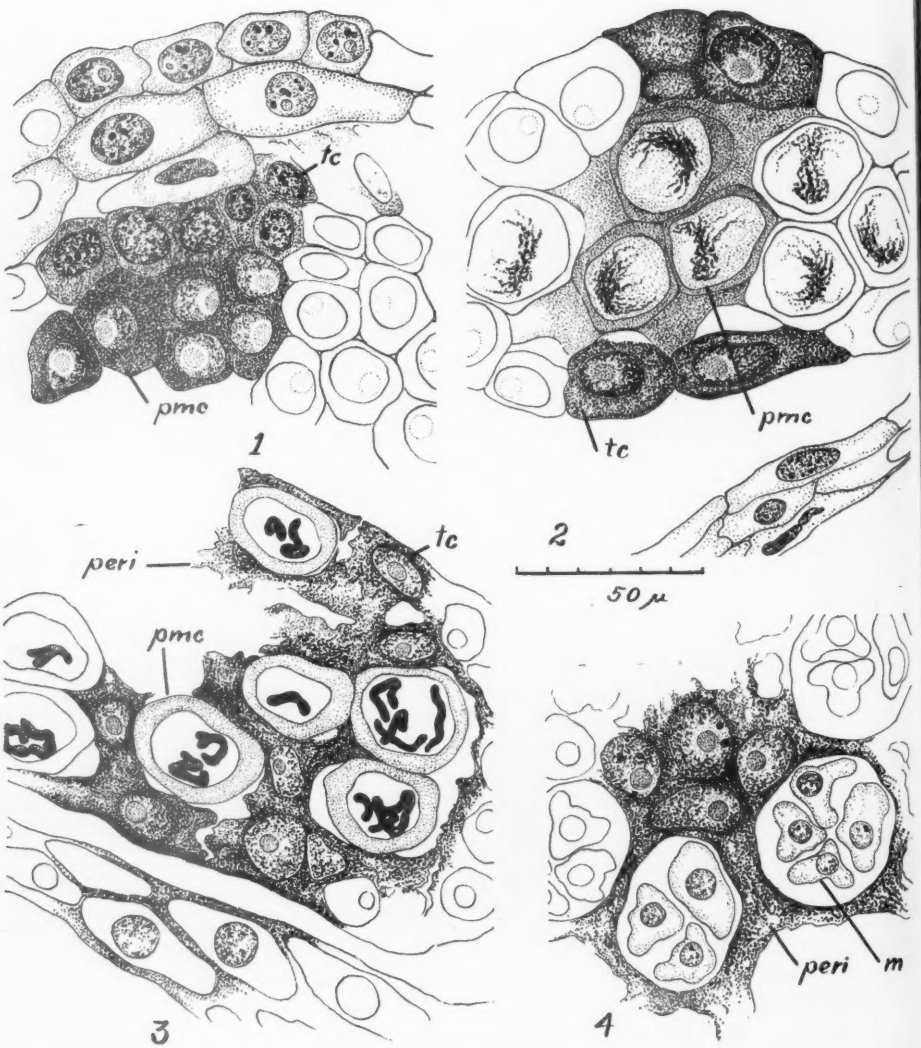
Observations

In general, the formation of pollen in *Rhoeo* is like that of other flowering plants. On the inside of the anther wall there is a single layer (usually) of tapetal cells which surrounds the pollen mother cells. In many plants the tapetal cells remain attached to the anther wall and undergo dissolution as the pollen develops, so that the latter is bathed in what has been commonly regarded as a nutritive fluid. In *Rhoeo*, however, the tapetal cells become detached from the anther wall first, and then, about the time of diakinesis in the pollen mother cells, the cell walls disappear in the tapetal tissue and a multinucleate syncytium or periplasmodium results. The latter is absorbed by the maturing pollen grains.

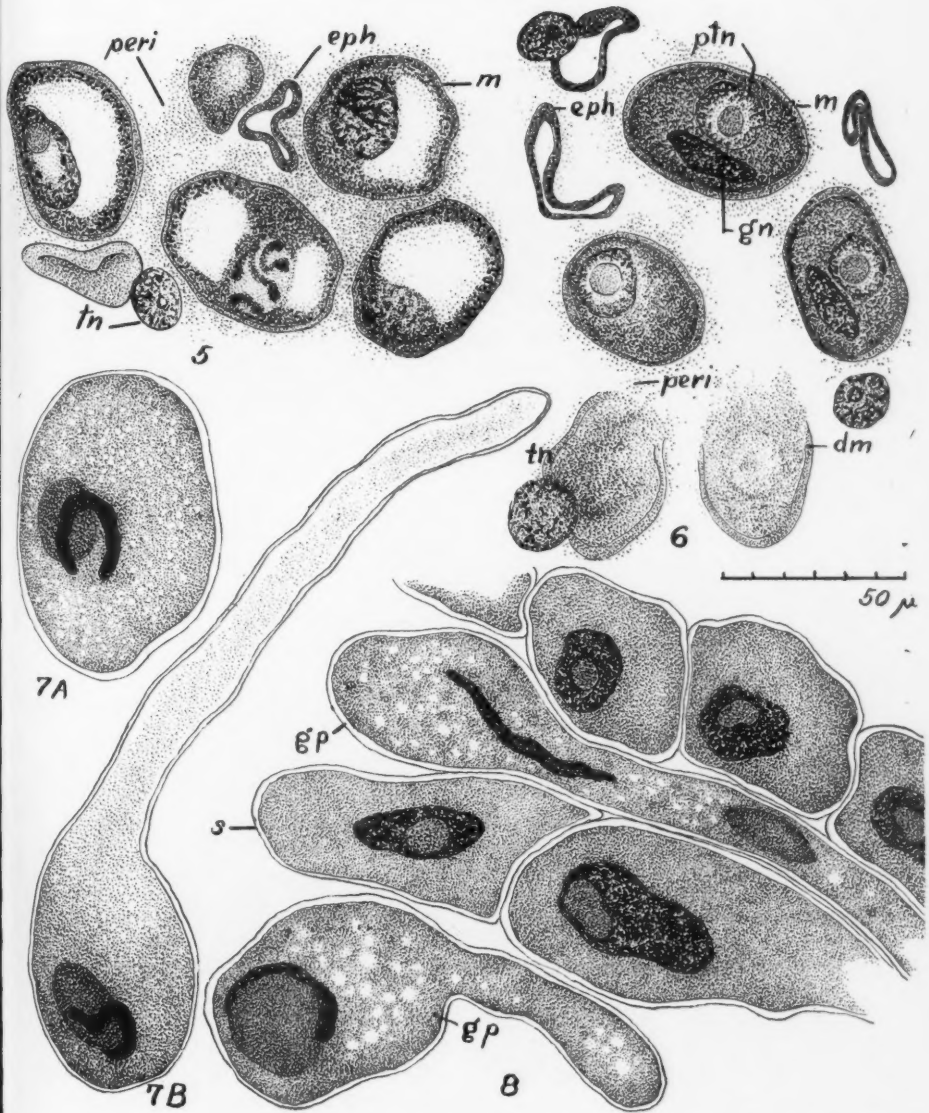
MEIOTIC GROWTH.—Figures 1–3 are sections through anthers showing several phases of this growth period. Three salient points are brought out by the drawings. First is the increase in the volume of the pollen mother cells (*pmc*). Second, in the earlier stage (fig. 1), the cytoplasm of these cells stains an intense red, but as growth goes forward this basophily almost or entirely disappears (fig. 3). In contrast, the cytoplasm of the tapetal cells (*tc*) retains its basophily throughout this period. Third, at about the stage represented in figure 3, the walls of the tapetal cells disappear and some of the tapetal nuclei show a loss of chromaticity, that is, are beginning to undergo dissolution.

During the two meiotic divisions the cytoplasm of the pollen mother cells is almost or entirely free of basophilic materials, and the four microspores which result occupy about the same space as the single pollen mother cell from which they came (fig. 4). That is, there is no appreciable growth in total volume during this period. After fixation with Helly's fluid the microspores usually appear shriveled, but after Navashin's fluid this artifact does not show.

POST-MEIOTIC GROWTH.—Figures 4–7 show steps in the growth of microspores.



FIGS. 1–4.—Fig. 1, section through young anther showing tapetal cells (*tc*) and pollen mother cells (*pmc*). Figs. 2, 3, sections through anthers showing two stages in growth of pollen mother cells before first meiotic division. Fig. 4, section showing very young microspores (*m*) immediately after the two meiotic divisions.



FIGS. 5–8.—Fig. 5, microspores (*m*) at stage when nuclei are preparing for division; tapetal nuclei (*tn*) and empty pollen hulls (*eph*) appear in periplasmodium (*peri*). Fig. 6, later stage in microspore growth showing normal pollen grains with pollen tube (*ptn*) and generative (*gn*) nuclei. Some microspores are degenerating (*dm*), and periplasmodial fluid is nearly all absorbed. Fig. 7*A*, *B*, mature and germinating pollen cells, respectively. Fig. 8, optical section through stigma showing thick-walled and very basophilic cells of stigma (*s*) and germinating pollen (*gp*) (from aceto-carmine whole mount).

At the outset (fig. 4) the cytoplasm of the microspores does not stain with pyronin or at most is a very light pink. Figure 5 is a later stage in which the microspores are much larger. This increase in size is due to growth in the size of the nucleus, to the volume of the cytoplasm, and to the development of a cell vacuole. At the same time the cytoplasm has become very basophilic. In the anthers studied, many of the microspores for some reason fail to develop completely; such cells undergo rapid dissolution, and empty cellulose hulls (*eph*) are a conspicuous feature in all the older anthers. The cytoplasm and many nuclei of the periplasmodium are largely dissolved by this time and, after fixation with Helly's fluid, give a fine fibrous coagulum which stains red to pink. In figure 5 several microspore nuclei show condensation of the chromosomes in preparation for the postmeiotic division, which will give the mature pollen grain two nuclei.

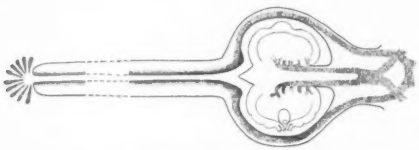


FIG. 9.—Semi-diagrammatic section through pistil showing distribution of cells which exhibit intense basophily of cytoplasm.

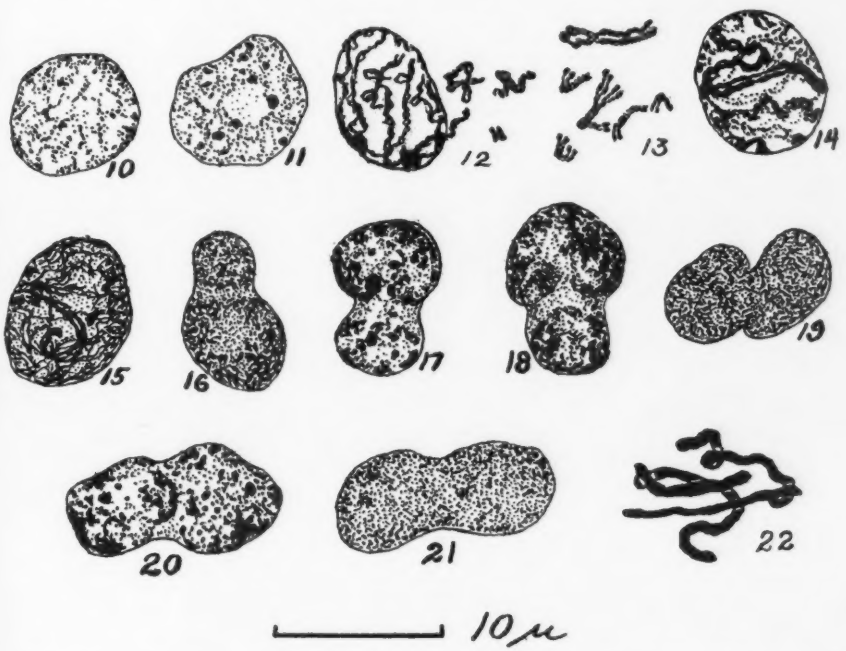
In figure 6 two normal pollen grains are shown. The cytoplasm has increased greatly in these cells, so that the cell vacuole is almost or wholly obliterated, and it stains deeply with pyronin. By this time most of the fluid of the periplasmodium has been absorbed and only a few tapetal nuclei remain. A fully mature pollen cell is shown in figure 7*A*. Such cells have increased in volume (as compared with the stage shown in figure 6), the generative nucleus has become greatly elongated, is often U-shaped, and the cytoplasm contains a number of round, light-refracting granules.

POLLEN-TUBE GROWTH.—When mature pollen grains are placed on the stigma of the flower they increase somewhat in volume and the cytoplasm stains less intensely. As the pollen tube is formed and penetrates the space between the cells of the stigma, its cytoplasm stains a bright pink, and this basophily persists for a long distance down the lumen of the style (figs. 7*B*, 8). The total volume increase in the germinating pollen cells is estimated as two to four times the volume (of the cytoplasm) of mature pollen grains.

There are definite areas in the pistil of the flower which may show heavy deposits of ribonucleic acid in the cytoplasm of cells. These are shown semi-diagrammatically in figure 9. The very large thick-walled cells at the surface of the stigma are about as basophilic as the mature pollen grains (fig. 8). A lumen passes from the loosely organized stigma down through the style to the ovary, where it divides so that each of the three carpels (only two shown) receives a branch. The pollen tubes thus have a passageway which gives them access to the micropyles of the ovules. The cells immediately surrounding the lumen in the style are small and the cytoplasm is very basophilic. Near the top of the ovary the basophilic tissue splits into three bundles which pass around the outer wall of the ovary to the pedicel. These bundles of tissue are closely associated with spiral vessels (not shown). A separate mass of basophilic cells arises in the stem and passes to the carpels, where numerous branches spread out into the tissue which is destined to form the greater part of the seed. The more exact details of the distribution of ribonucleic acid in the ovary are now being studied. Incidentally, oxalic acid crystals, often

present in the walls of the ovary, stain brilliantly with pyronin.

TAPETAL TISSUE.—In very young flower buds (fig. 1) the anther wall is composed of large vacuolate cells, the cytoplasm of which stains a light pink color with pyronin. Normal mitoses are in figure 1, in contrast to the cells of the anther wall, the cells are much smaller, no cell vacuole is visible, there are much heavier deposits of chromatin in nuclei, and the cytoplasm stains deeply with pyronin. Nucleoli are either small or entirely absent at this time. As the pollen



FIGS. 10–22.—Various endomitotic division stages in tapetal nuclei. Figs. 10, 11, resting stages. Figs. 12, 13, split and coiling prophase chromosomes. Figs. 14, 15, maximum degree of condensation of chromosomes observed. Figs. 16–21, constriction of nuclear wall following condensation of chromosomes. Fig. 22, normal somatic metaphase showing size of dividing chromosomes.

often found. Although in the diploid tissues of *Rhoeo* two nucleoli are seen, one larger than the other, at the stage shown in figure 1, these are inconspicuous in the anther wall. Heterochromatic granules are always prominent; two pairs are quite large and there are a number of smaller bodies. As the anther grows the cells in the wall increase in number by mitoses and the cellulose walls become rather thick (fig. 3).

In the tapetal layer at the stage shown mother cells increase in size (fig. 2) some of the tapetal cells and their nuclei increase greatly also and nucleoli become prominent. Since growth of the pollen mother cells involves the reduplication of the chromosomes, the question arises: Is the growth of the tapetal cells accompanied by a similar doubling of the chromosomes? COOPER (9) has reported that in *Rhoeo* the nucleus may divide mitotically one or more times, and if the resulting nuclei do not fuse multinucleate cells

result. In the writer's material no multinucleate tapetal cells have been observed, nor has there been any case in which the nuclear wall has been broken down. On the other hand, various normal prophase stages are common, showing that the chromosomes are condensing by coiling, and dumbbell-shaped nuclei are frequent (figs. 10–21). Such figures suggest that, in addition to the normal mitoses and fusion observed by COOPER, tapetal nuclei also increase in volume by an intranuclear, or endomitotic, division cycle.

Discussion

The preceding observations are of general interest because of the light they throw on the questions: What is the immediate source of the nucleic acids which appear in pollen mother cells or their derivatives; What roles do cytoplasmic nucleic acids play in growing cells; What is the relation between cytoplasmic and nuclear nucleic acids?

At the outset it is desirable to have in mind some basic facts about nucleic acids. These are made up of nucleotides, each of which contains phosphoric acid linked with a definite type of sugar, which in turn is linked with a nitrogen-bearing purine or pyrimidine base. The conventional formula for ribose nucleic acid is the tetranucleotide:

Phosphoric acid–ribose sugar–adenine (purine)
Phosphoric acid–ribose sugar–cytosine (pyrimidine)
Phosphoric acid–ribose sugar–guanine (purine)
Phosphoric acid–ribose sugar–uracil (pyrimidine)

In animals and plants two types of nucleic acids are commonly found, desoxyribose and ribose, which differ chemically in a slight change in the sugar component. In chromatin, or thymonucleic, or desoxyribose nucleic acid, as it is variously called, an H is substituted for an OH group in the ribose sugar giving desoxyribose sugar. In addition to the difference in the sugar, chromatin differs from ribonucleic acid in the following ways: (*a*) No desoxyribose nucleotide is known which enters into the makeup of a coenzyme, whereas all nucleotides known to act as coenzymes have the ribose sugar; in this sense chromatin may be spoken of as an enzymatically inactive form of nucleic acid. (*b*) Desoxyribose nucleotides are easily polymerized into very large molecules, and the evidence from ultra-centrifuge and X-ray determinations indicates that chromatin has about 2000 such nucleotides linked together; evidence for such high polymers of ribose nucleotides is not well established. (*c*) Chromatin is normally confined to the nucleus, while ribose nucleic acids are predominantly cytoplasmic in location.

There is considerable direct and indirect evidence that in living cells of both plants and animals one form of nucleic acid is easily changed into the other; indeed, WHITE (19) has suggested that there is such an exchange during each mitotic cycle, a conclusion which seems to be supported by the present study on *Rhoeo*. MASING (10), using quantitative chemical methods, showed that there is as much nucleoprotein (measured as phosphorus combined with protein) in the unsegmented egg of the sea urchin as in the larva when it hatches, and from this he concluded that there had been no synthesis of nucleoproteins during cleavage. Later, BRACHET (1) demonstrated that the nucleoprotein of the unsegmented egg of the sea urchin is in the form of ribonucleic acid. During cleavage the amount of ribonucleic acid falls off as the quantity of thymonucleic acid increases, and BRACHET concluded that the ribose was partially converted into the desoxyribose type during cleavage.

That thymonucleic acid is readily changed into the ribose type is indicated by two lines of evidence. CASPERSSON

and SCHULTZ (**7**) found that there is less ribose nucleic acid in the eggs of XX females of *Drosophila* than in females of the XXY type, the extra nucleic acid being brought into the egg by the extra Y chromosomes of the nurse cells. Again, in the germinal vesicle of the eggs of frogs and toads, during the early growth of the oocytes granules of heterochromatin become detached from the chromosomes, migrate to the surface of the nucleus, and there act as centers for the elaboration of ribonucleic acid which accumulates in large quantities just outside of the nuclear wall. During this process the heterochromatin disappears, presumably being used up or somehow converted into the ribose form (**3, 15, 16**).

The meiotic growth period throws no light on the source of the ribonucleic acid in the cytoplasm of pollen mother and tapetal cells, because in the youngest anthers examined large amounts of this basophilic material are already present. The growth of the pollen mother cells at this time seems to center mostly in the nucleus, since the nucleo-cytoplasmic ratio shifts from about 1:2 to 1:1. In preparation for the meiotic divisions, the chromosomes are reduplicated once, perhaps twice, and the fact that during this period the ribonucleic acid disappears from the cytoplasm suggests that it is transferred to the nucleus and enters into the formation of the chromosomes as thymonucleic acid. On the other hand, in some of the tapetal cells the chromosomes must have been reduplicated, since some cells attempt to divide, but so far as can be determined the cytoplasm of such cells shows no decrease in basophily. It seems possible that in these cells—which are destined to undergo cytolysis—the nucleic-acid metabolism is disturbed, just as when proliferating cells are irradiated (**11**).

During the meiotic divisions, which go forward without an intervening resting stage, there is little or no basophilic material left in the cytoplasm, and the very young microspores contain practically none of this material. Concomitant with the onset of growth in the microspores, ribonucleic acid appears in the cytoplasm, and—if the degree of basophily is a reliable index—its concentration steadily increases and reaches its maximum in the mature pollen grains. Where does this nucleic acid come from? Either it is derived from the chromatin of the microspore nucleus or it is synthesized from the fluid in which the microspores are bathed. The first seems unlikely, since after the two meiotic divisions the microspore nucleus is less chromatic than at any other period. On the other hand, the fluid in which the microspores are bathed is derived from the dissolution of tapetal cells and of microspores which fail to complete development, and both of these sources are very rich in ribonucleic acid. Although little is known as yet about the steps involved in the transfer, that is, how far the polymerized ribose nucleotides are broken down, it seems a safe inference that the anther fluid is the source of the nucleic acids which accumulate in the cytoplasm of microspores which become mature.

If ribonucleic acid is essential for growth, why is so much of it present in mature pollen grains when growth is at a standstill? In answering this question it may be pointed out that the history of the tapetal cells and of the development of pollen has a close parallel in animal eggs formed through the agency of nurse cells, and fundamentally the ends subserved seem the same in both cases. In *Drosophila*, for example, the oocyte grows at the expense of fifteen nurse cells. The latter become very large, are high polyploids containing large amounts of chromatin and much ribonucleic acid

(14, and unpublished data). By the time the oocyte becomes mature the substances in the nurse cells have been completely absorbed in the egg cytoplasm, which is now rich in ribonucleic acid. In the same way in *Rhoeo*, as the pollen matures, the tapetal and degenerating germ cells are completely absorbed. Apparently the reason so much nucleic acid is deposited in the cytoplasm of animal eggs is that it is needed for the rapid synthesis of proteins and other complex substances as the oocyte and embryo develop and as a source of nucleic acids for the formation of cleavage nuclei (13). Similarly, in the case of the pollen in *Rhoeo*, large amounts of cytoplasmic nucleic acid are needed for the extremely rapid elaboration of protoplasm during the formation of the pollen tube.

It seems possible that the abundant ribonucleic acid in the style of the flower along the path which the pollen tube follows insures an ample supply of food material for the growing pollen tube. The basophilic tissue which passes from the pedicel of the flower into the carpels, where rapid synthesis of new materials takes place, emphasizes the relation between growth and the presence of ribonucleic acid, although it is not clear why cells far removed from regions of most rapid growth should be so basophilic. It is significant, perhaps, that these cells are associated with the spiral vessels which carry water to the growing tissues and suggests that other substances may be transported.

While it is clear that ribonucleic acid is essential for cell growth, the way it functions is not well understood. It has long been recognized that enzymes play dominant roles in the chemistry of the cell. And the fact that so many of the known enzymes contain ribose nucleotides has led a number of workers to suggest that the origin of these are the cytoplasmic nucleic acids so abundant in rapidly synthesizing cells. This explanation receives support from the fact that a nucleotide containing adenine is found in coenzymes 1 and 2 and adenylic acid. OSTERN, TERSZAKOWEC, and HUBL (12) were able to change ribonucleic acid into adenosine, *in vitro*, and have interpreted their results as a step in the formation of adenylic acid. CASPERSSON and BRANDT (6) point out that, while probably (though as yet unproved) coenzymes come from ribonucleic acids, there is far more of the latter present in the cytoplasm than would be needed to catalyze chemical reactions, and they think the ribose nucleotides play other fundamental roles in growth. There is one possibility which has not yet been stressed.

Since nucleic acids were first found in nuclei it is natural to consider new nucleotides as being synthesized at this site in the cell. On the other hand, many facts about the mitotic cycle can best be understood by assuming that new nucleotides are synthesized first in the cytoplasm and are then passed somehow into the nucleus. On this assumption a mitotic cycle might involve the following changes. During the resting stage CASPERSSON (4) found that the total amount of nucleic acid in the nucleus is at a minimum, whereas it is known (17) that cytoplasmic activity including growth and metabolism is at a peak, presumably because of the presence of ribose nucleotides which are essential for growth. When growth is complete and the prophase begins, ribose nucleotides pass into the nucleus and are there converted into desoxyribose nucleotides and carried in a compact, highly polymerized form on the chromosomes over into the two daughter cells. This migration of nucleotides (however it may occur) into the nucleus ex-

plains the sudden increase in nucleic acid which CASPERSSON observed in the nucleus, a fact which in the past has been interpreted to mean that new nucleotides were being synthesized in the nucleus from relatively simple components. During the pro-metaphase stages cytoplasmic activity is at a low ebb (17), presumably because of the absence of ribonucleotides (coenzymes). After the telophase, the decrease in the amount of nucleic acid in the nucleus is due to the passage of some of it into the cytoplasm, where it is converted into ribose nucleotides, there to implement protein growth and the synthesis of new nucleotides, and perhaps to perform other functions not now recognized.

This view of nucleotide exchange between nucleus and cytoplasm is (except for minor details) essentially that expressed by WHITE (19) and is supported by evidence from diverse fields of investigation. SCOTT (18), studying mitosis by the microincineration method, found that in the resting stage the inorganic ash, consisting mainly of calcium and magnesium residues, was evenly distributed in the cell. In the prophase of mitosis these salts pass into the nucleus and are deposited on the chromosomes. The simultaneous passage of nucleotides and of calcium and magnesium ions into the nucleus suggests that the latter may play some role in the attachment of these nucleotides to the protein framework of the chromosomes.

MITCHELL (11) has found that when growing and differentiating cells are irradiated with X- or gamma rays, during the period when mitoses are inhibited, there is an accumulation of ribose nucleotides in the cytoplasm of the cells. Quantitative measurements indicate local concentrations of as much as 3 per cent. During this time there is no increase in the amount of nucleic acid in the nucleus. MITCHELL explains these facts as "probably due to inhibition by the radiation of the process of reduction of ribonucleotides to desoxyribo-nucleotides in the nucleus."

If ribose nucleotides of the resting stage are in part precursors of chromatin in rapidly growing and dividing cells, this would account for some of their concentration in the cytoplasm. Furthermore, as in other organic systems, the two types of nucleic acid would be expected to be in some sort of equilibrium, and this would explain the presence of large amounts of chromatin in the nucleus when the cytoplasm shows large amounts of ribose nucleotides.

Attention should be drawn to the limitations of the methods employed in this study. Basophily in the cytoplasm depends on the presence of free acid groups, and oxalic-acid crystals, which often show in the walls of the ovary in *Rhoeo*, stain as brilliantly as the ribose nucleotides. When the basophily is removed from the cytoplasm by treatment with the ribonuclease enzyme, the conclusion is warranted that polymerized ribose nucleotides were present initially. The absence of basophily in the first place, however, does not indicate necessarily the absence of ribonucleotides but the absence of ribonucleotides with uncombined or free acid groups. Obviously the methods employed are useful only in species in which the ribonucleic acid can be stained with basic dyes.

Summary

1. A cytoplasmic basophily, which is removed when cells are treated with a ribonuclease enzyme, has been used as a means of identifying ribose nucleic acids and of following their distribution in the cells of the anthers of *Rhoeo*, and to a

lesser extent in the pistil of the flower. Rapid cell growth is invariably accompanied by large amounts of ribonucleic acid in the cytoplasm and heavy deposits of chromatin in nuclei.

2. In young anthers the cytoplasm of both tapetal and sporogenous tissues is extremely rich in ribonucleic acid. As the pollen mother cells prepare for the meiotic divisions, the ribonucleic acid disappears from the cytoplasm, presumably being passed into the nucleus and changed into desoxyribose nucleic acid (chromatin). Initially the cytoplasm of microspores is devoid of basophilic material, but as they grow into mature pollen grains large amounts of ribonucleic acids accumulate in the cytoplasm.

3. The growth of tapetal cells is accompanied by one or more endomitotic nuclear division cycles. The tapetal cells, which are rich in cytoplasmic nucleic acid at the time of cytolysis, are the source of the ribonucleic acid which accumulates in the maturing pollen grains.

4. There is a similarity between microspore growth at the expense of tapetal cells and growth of oocytes in animals through the agency of nurse cells. Large amounts of ribonucleic acid are stored in mature pollen grains and used in the extremely rapid synthesis of new materials which accompanies pollen tube growth.

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HISTOLOGICAL COMPARISON OF CUCUMBER FRUITS DEVELOPING PARTHENOCARPICALLY AND FOLLOWING POLLINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 554

J. O. YOUNG

Introduction

Parthenocarpy in the cucumber has long been recognized by horticulturists, but it was first made the subject of botanical investigation in 1902 by NOLL (15), who defined the term and observed fruits containing abortive seeds with undersized seed coats. Two years later KIRSCHNER (13) reported a doubtful case in which 78 out of 95 seeds produced in a single unpollinated cucumber were viable. HARTLEY (12) reported the stimulation of fruit development in tobacco by treating the stigmas with *Azalea* pollen, corn flour, magnesium sulphate, or air-slaked lime. FITTING (4), YASUDA (20), and GUSTAFSON (10) have achieved similar results with pollen extracts.

Since the development of the growth-substance concept, several investigators, notably GUSTAFSON (8, 9), GARDNER and MARTH (6), and WONG (19), have succeeded in inducing fruit development by growth-promoting chemicals. These investigators worked chiefly with species of the Cucurbitaceae and Solanaceae, as well as with holly and strawberry. GARDNER and KRAUS (5) made a histological study of artificial parthenocarpy in the holly, noting that development closely parallels that of pollinated fruits, except for the disintegration of the megagametophyte and the absence of embryo and endosperm. VAN OVERBEEK *et al.* (18) reported the formation of pseudo-embryos in *Datura* fruits which had been injected with auxin.

TUKEY (17) showed that living embryos are essential for development of cherry and peach fruits. He correlated his findings with the three stages in the growth curves of these drupe fruits. Using the *Avena* test method, GUSTAFSON (7, 11) reported that auxin concentration of fruits is highest in developing ovules and associated tissues, and that it is higher in normally seeded than in parthenocarpic fruits of the same species. He concluded that auxins play a major role in fruit development.

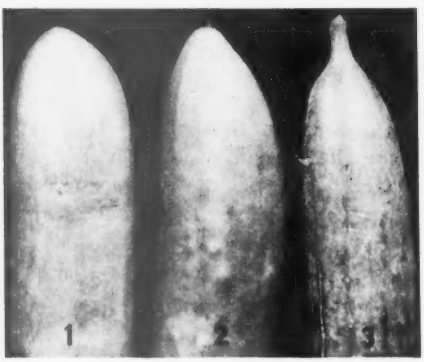
Certain varieties of cucumber regularly produce fruits without pollination. The results obtained with the variety Rollison's Telegraph, which exhibits natural parthenocarpy, are given here.

METHODS.—Seeds were planted February, 1941, in rich garden soil and grown on a well-lighted greenhouse bench. The plants were kept watered at all times but no attempt was made precisely to control the temperature or the humidity. During the later stages of the experiment the plants were supplied with complete nutrient solution high in nitrate to insure continued vigorous growth. The vines were trained upon a horizontal trellis about 7 feet above the floor level, and the developing fruits were allowed to hang without support.

Uniform pistillate flowers were selected at two developmental stages and treated with 2 per cent indoleacetic acid in lanolin paste. Controls were treated with pure lanolin. One lot was treated at full bloom and a second lot at approximately 4 days before full bloom. At the

time of anthesis the ovaries used ranged from 3.5 to 4.5 cm. in length. Larger or smaller ovaries were discarded. Four days before full bloom the developing ovaries were 1.8–2 cm. long and the still-green petal lobes extended about 4 mm. beyond the calyx.

The upper portion of the perianth, including the stigma and staminodia, was removed by a transverse cut through the nectary, and the indoleacetic acid in lanolin paste was spread in a thin layer over the freshly exposed surface. The lower part of the floral tube remained attached to the ovary by the narrow neck about 1 mm. long. Pure lanolin was used for controls. A third lot of flowers was hand-pollinated, and a fourth was allowed to develop without treatment.



FIGS. 1–3.—Apical portions of fully developed fruits: Fig. 1, pollinated; untreated fruits and controls treated at full bloom were similar in appearance. Fig. 2, treated with indoleacetic acid at full bloom; fruits treated with pure lanolin 4 days before full bloom were similar in appearance. Fig. 3, treated with indoleacetic acid 4 days before full bloom, showing apical tumor.

Portions of the fruits were fixed in Navashin's fluid and transferred to paraffin by the tertiary butyl-alcohol method. The material was transferred from the fixing fluid directly to a mixture containing ethyl alcohol 50 per cent, tertiary butyl-alcohol 20 per cent, and water 30 per cent. This procedure gave as good results as the more laborious method of washing in water and running through a longer series of changes. Sections were cut at 10 μ and stained in safranin, gentian violet, and orange G.

Gross development

Virtually all the ovaries, including those untreated, developed rapidly for several days. Toward the end of the experiment, however, the presence of large developing fruits inhibited early growth of other fruits on the same vine. Fruits treated with the acid 4 days before full bloom were decidedly smaller than any of the others.

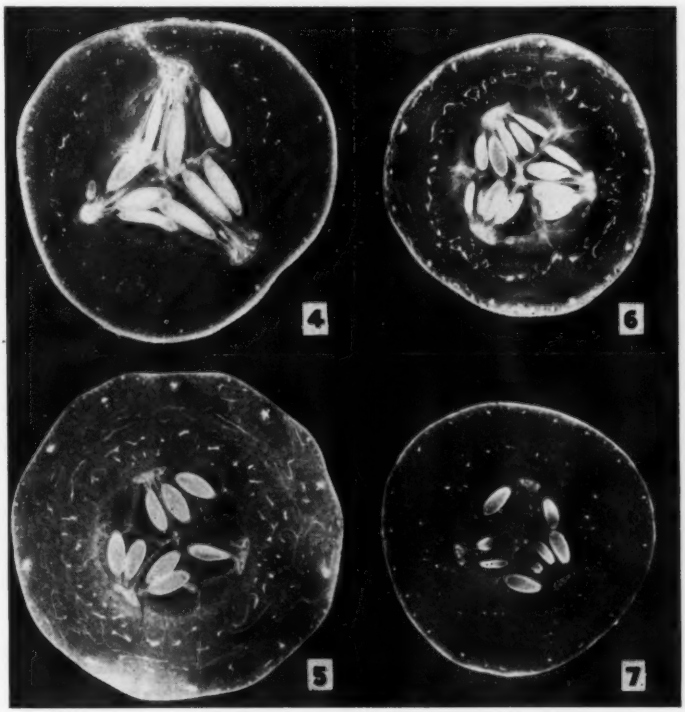
POLLINATED FRUITS.—Within a day or two after pollination the perianth and included structures began to wither, and usually they were completely dried within a week. These dead tissues usually fell away and left a smooth rounded scar (fig. 1). At the end of 20 days many of the ovules had attained a length of 12 mm. and the hardened seed coats inclosed well-formed embryos (fig. 4).

UNTREATED FRUITS.—In external appearance the untreated fruits developed identically with the pollinated fruits. Some of the ovules attained full size, but more frequently they were slightly smaller (fig. 5) and the seed coats were often sunken on the flattened sides. In no cases were embryos found.

FRUITS TREATED AT FULL BLOOM.—Fruits treated with indoleacetic acid at full bloom differed from the untreated controls in that the apical tissues below the cut surface remained alive until harvested and sometimes increased slightly in size, resulting in a small protuberance at the tip of the mature fruit (fig. 2). The ovules were slightly smaller than those of the untreated fruits (fig. 6). In corre-

sponding controls treated with pure lanolin the neck and perianth tissues dried out in the same manner as those on pollinated or untreated plants.

FRUITS TREATED 4 DAYS BEFORE FULL BLOOM.—Fruits treated with indoleacetic acid 4 days before full bloom developed characteristic apical tumors, involving the tissues of the nectary, floral tube, and the neck. The tumors reached a maximum diameter of slightly more than 1 cm. and a length of nearly 2 cm. (fig. 3). At the time of treatment the nectary tissue was light yellow and the lower part of the floral tube only slightly green. During the first 2 days after treatment the tissues of the floral tube and neck became lighter in color and a slight swelling was observed. After 4–5 days the tumors began to develop a bright green color throughout. Subsequent growth of the tumors was more rapid, and maximum size was usually attained in 10–15 days. The tissues of the nectary proliferated more extensively than those of the floral tube and greatly overtopped the latter structure. In no case did the tumors develop the large irregular masses reported for stems and fruits of the bean (14). No root primordia were observed. Ovules attained a final size decidedly smaller than those of the controls (fig. 7). Hardening



FIGS. 4–7.—Cleared cross sections of fully developed fruits: fig. 4, pollinated, showing embryos; fig. 5, untreated; fig. 6, treated with indoleacetic acid at full bloom; fig. 7, treated with indoleacetic acid 4 days before full bloom.

of the integuments was markedly inhibited and restricted to the micropylar end of the ovule.

The apical portions of controls treated with pure lanolin at 4 days prior to full bloom remained intact for several days. At the end of 24 days, tissues of the floral tube, nectary, and part of the neck were dead but no periderm or scar tissue had formed. At this time the gross appearance was similar to that of fruits treated with indoleacetic acid at full bloom (fig. 2), except that there was more dead apical tissue.

Histological details

FERTILIZED OVULES.—No comprehensive published account of ovule development in the cucumber has been found. TILLMAN (16) investigated the megagametophyte development and embryology. She noted the massive nucellus, the swelling of the pollen tube, digestion of cells within the nucellar beak, and the attainment by the ovules of a strictly anatropous position. The findings of the writer are similar in all major respects. FICKEL (3) showed that the major portion of the seed coat is derived from the outer integument, the inner integument attaining a thickness of only two cell layers. BARBER (1) added a few details of cell size and arrangement but erroneously figured an inner integument several cells in thickness early in development.

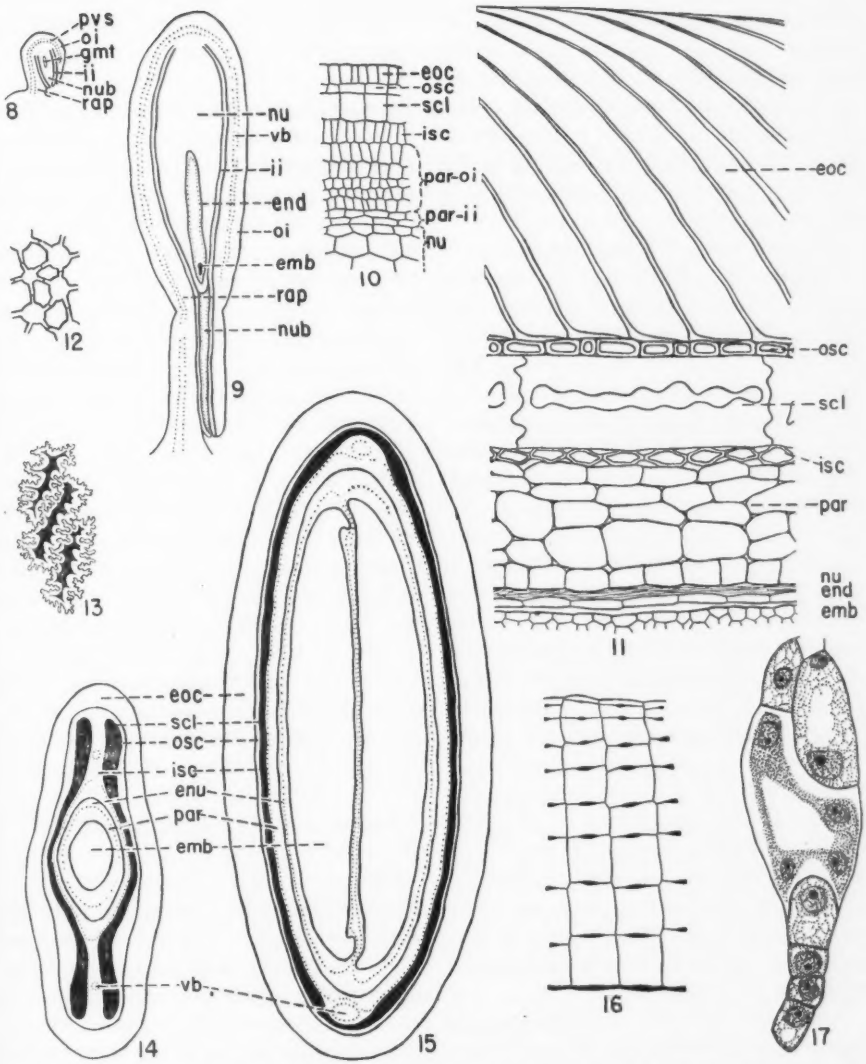
Four days before full bloom the megaspore mother cell may be clearly distinguished two to four cell layers from the tip of the nucellus. Continued tangential division of the overlying cells results in the development of a long nucellar beak, which is discernible at the time of anthesis. The inner integument develops as a covering two or three cells in thickness, which finally closes over the nucellar beak. The outer integument arises as a more massive layer and may be eight cells in thickness by the time of full bloom. A vascular strand extends through the raphe to the chalaza and is continuous with a strand which extends through the outer integument as far as the base of the nucellar beak (fig. 8).

At full bloom meiosis has been completed and the gametophyte is partially or fully developed. It occupies a position just proximal to the base of the nucellar beak. At this stage mitotic activity is evident in the integuments and at the chalazal end of the nucellus.

Fertilization is accomplished in ovules near the apical end of the ovary within 72 hours after pollination. Just before fertilization the pollen tube enlarges in the proximal portion of the nucellar beak and nucellar cells in this region are digested. Soon after fertilization, cells of the nucellus adjacent to the embryo sac become meristematic, and several mitotic divisions occur. Eight days after full bloom the embryo is a small, slightly elongated cellular mass (fig. 9). At this stage mitotic activity is still in progress but all tissues of the seed coat are clearly defined.

Subsequent development of the seed coat involves differentiation and maturation of the several tissues. Comparison of figure 10 (8 days from full bloom) and figure 11 (20 days after full bloom) brings out the development of tissue types in the seed coat.

The seed coat may be compared to a rigid laminated sac of many layers. In the mature seed, cells of the innermost layer are parenchymatous. This tissue is of mixed origin. The inner portion derives from the inner integument and the remainder arises from the inner four or five tiers of cells of the outer integument. As the seed coat matures the exterior tissues increase in size more than the interi-



FIGS. 8–17.—Fig. 8, longisection of ovule at full bloom; ×35. Fig. 9, same, 8 days after full bloom; ×35. Fig. 10, portion of longisection through seed coat 8 days after full bloom; ×350. Fig. 11, same, 24 days after full bloom; ×350. Fig. 12, stellate cells from tissue surrounding vascular bundle; ×450. Fig. 13, elongated stone cells from macerated seed coat; ×200. Fig. 14, cross section of mature seed near hilum; × 35. Fig. 15, same, through center of seed; ×35. Fig. 16, cells of outermost layer of seed coat as seen in cross section through seed, showing thickenings on cell walls; ×350. Fig. 17, anomalous development of megagametophyte: four cells at lower part of figure are at micropylar end; ×750 (*emb*, embryo; *end*, endosperm; *enu*, endosperm and nucellus; *eoc*, elongated outer cells; *gmt*, megagametophyte; *ii*, inner integument; *isc*, inner layer of thick-walled derivatives of stellate cells; *nu*, nucellus; *nub*, nucellar beak; *oi*, outer integument; *osc*, outer layer of thick-walled derivatives of stellate cells; *par*, parenchyma; *pvs*, provascular strand; *rap*, raphe; *scl*, elongated stone cells; *vb*, vascular bundle).

or parenchyma. The interior cells thus become flattened and are pulled from their original positions, so that at maturity the parenchymatous layer is about five cells in thickness. Intercellular spaces are formed and the identity of the inner integument as a distinct tissue is lost.

External to the parenchyma is a layer of stellate cells. This layer is composed of a single tier of cells on the flattened sides of the seed, but on the lateral margins it is many cells in thickness. The vascular bundle which extends around the seed is imbedded within this marginal layer of stellate cells. In the region of the vascular bundle little growth occurs and the stellate condition becomes best developed (fig. 12). At maturity each cell has many projections which extend in various directions and abut on adjacent projections from other cells. The tissue thus formed is lattice-like in appearance, the large intercellular spaces being merged to form a continuous three-dimensional labyrinth. Where this tissue is only one tier in thickness the cells become flattened as the seed coats enlarge. Thickening and hardening of the cell walls begins after the twelfth day from full bloom. This is first evident close to the base of the nucellar beak and proceeds rapidly to the chalazal end of the seed. No sclerenchyma is developed within the nucellar beak.

Outside the stellate cells is a layer of large stone cells which are greatly elongated in the longitudinal plane of the seed axis. Examination of macerated tissue shows these cells to be roughly rectangular in surface view, but the margins are deeply and irregularly incised (fig. 13). The lumen of each cell is narrow and branched and thus appears to be discontinuous when the seed is cut in median longitudinal section. In a few cells, however, the continuity of the lumen is clearly discernible (fig. 11). The cells of this tier are tightly dovetailed together, forming a hard impermeable layer. Near the nucellar beak this layer does not extend completely around the seed (fig. 14). In this region the tissue is in the form of two bands over the flat sides of the seed but not around the margins. Lateral to the vascular bundles this layer is two to four cells in thickness. The discontinuity of this layer around the hypocotyl permits the emergence of the embryo at germination when the empty seed coats remain intact.

The next layer is another tier of stellate cells. In the distal end of the seed coat this tissue is continuous with the inner layer of stellate cells (fig. 14). A cross section through the center of the seed, however, shows that here there are two layers of stellate cells separated by the tier of large stone cells already described (fig. 15). In the outer layer the stellate condition is practically lost when the cell walls thicken, but the various stages show that these cells are derived from the stellate type.

The outermost layer of the seed coat is composed of very elongate cells with characteristic thickenings on the inner radial walls. These cells are radially elongated 8 days after full bloom. As lengthening and maturation continue they become appressed, with the outer ends lying in the direction of the chalaza. Cross sections through the seed show that these cells develop in an orderly spatial arrangement. Since these cells overlap for most of their length, it appears in cross-sectional view that there are several distinct layers (fig. 16).

The elongated cells of this outer layer are continuous over the beak but lack thickenings in this region. The remainder of the beak is composed of a few stellate

cells and parenchyma. A constriction occurs at the base of the beak, and when the seed is broken away a considerable portion of the raphe, integuments, and nucellar remnants is lost (fig. 9).

In the mature seed the nucellus has been reduced to a membrane of collapsed cell walls, and a single layer is all that remains of the endosperm.

UNFERTILIZED OVULES.—Many stages in meiosis and megagametophyte development were carefully observed, but no deviations from the normal were found subsequent to treatment with indoleacetic acid, even when the application was made before the onset of the meiotic process. Three days after full bloom, however, the absence of the pollen tubes in ovules of unpollinated fruits was noted and the cells of the nucellar beak were not digested. No renewal of meristematic activity in nucellar cells adjacent to the megagametophyte occurred, as is the case when young embryos are present. In other portions of the ovule, divisions continued until 8 days after full bloom, or about as long as they do following fertilization.

The megagametophytes of the parthenocarpic fruits were occasionally observed to enlarge considerably. This sometimes continued for as long as 8 days after full bloom, but more often degeneration began about the fifth day. The fusion nucleus and the egg were usually the last recognizable remnants of the megagametophyte. These were sometimes discernible 16 days after full bloom. Even in the absence of an embryo, the nucellus developed into a massive tissue which completely filled the integuments. The cavity left by the degenerating gametophyte was often extended by cellular disintegration in the center of the nucellus.

Proliferation of gametophytic cells was observed in a single case. In a section of an ovule fixed 8 days after prebloom treatment with indoleacetic acid the condition shown in figure 17 was clearly seen. The free nuclear tissue is suggestive of endosperm and apparently has arisen by divisions of the fusion nucleus. The four cells at the micropylar end of the figure may have been derived from the egg. Since the antipodals have been observed to degenerate early in this variety, it would seem most probable that the two large cells at the chalazal end are of nucellar origin.

In the ovules of all fruits treated with indoleacetic acid and in the controls cell division continued long enough so that the pattern of the mature seed coats was definitely established. When the acid was applied 4 days before full bloom, hardening of the seed coats was greatly reduced and usually did not extend more than halfway to the chalazal end of the seed. When treatments were made at full bloom, hardening proceeded somewhat further, while in the controls the integuments sometimes reached a stage of development nearly equal to that of ovules containing embryos.

The preceding description is based on the most completely developed ovules, which usually are found near the apical end of the fruit. As a rule the ovules near the stem end are progressively smaller, and many of them never exceed 1–2 mm. in length.

APICAL TISSUES.—Following pollination, the stylar canal tissue is digested or disrupted within 48 hours by the developing pollen tubes. The adjacent intact cells become elongated in a radial direction, but meristematic activity is not initiated in any part of the floral tube, nectary, stylar tissue, or neck. There was some increase in the thickness of cell walls in the peripheral parenchym-

atous cells of the neck at 4 days after full bloom. By this time the nectary tissue has degenerated and a periderm may have begun to form at the base of the neck. At 8 days after full bloom the periderm is well formed, and on its outer surface cell walls are appreciably thickened. All the distal dried-out tissues may now fall away, or they may remain attached. At 20 days after full bloom pitted stone cells were found abundantly in the tissue immediately beneath the apical periderm. Apical tissues of untreated plants and of the controls treated with lanolin at full bloom dried out in much the same manner, except that the changes incident to penetration of the pollen tubes did not occur and a periderm developed across the tissue of the stylar canal.

Following treatment with indoleacetic acid at full bloom, disintegration of the apical tissues was greatly retarded. At the end of 20 days the tissues of the nectary and floral tube were usually shriveled, but cells of the neck were alive. A periderm was not generally formed at the base of the neck, but tangential cell divisions were commonly observed in the peripheral neck region. Cell walls in this zone often became somewhat thickened, with conspicuous pits, but no stone cells or fibers were found. There was evidence of slight proliferation throughout the parenchymatous cells of the neck.

Earlier than 4 days before full bloom meristematic activity was abundant in the tissues of the neck. New cell walls were formed, chiefly in the transverse plane of the axis, so that the cells of the parenchyma presented a more or less tabular or ladder-like appearance. In lanolin controls 4 days before full bloom this pattern was recognizable as long as 24 days later. Except for moderate increase in cell size, the tissues of the neck remained much as they were at the time of treatment. The cells of the nectary and floral tube gradually degenerated. Only slight thickening of cell walls was observed, and no sclerotic or vascular tissues were differentiated subsequent to pre-bloom treatments with pure lanolin paste.

When the 2 per cent acid-lanolin mixture was applied 4 days before full bloom, there was a prompt response in the floral tube, nectary, and neck. Cells immediately below the cut surface of the nectary were slightly enlarged after 24 hours; the nuclei were larger and showed greater affinity for the stains; the cytoplasm increased in amount and was often extended across the large central vacuoles. Apparently normal cell divisions occurred. Continued increase in cell size was chiefly in the direction of the fruit axis, so that markedly elongate cells were formed. By repeated mitotic divisions these cells were divided into groups of smaller cells. The outlines of the parent cell walls were often discernible for several days, much as described in stems of *Lilium* by BEAL (2). In the nectary of the cucumber, however, the new walls were all formed in a single plane, so that in longitudinal section the entire unit of derived cells appeared as a single row. After the initial activity, mitotic divisions gradually decreased and stopped. Cell enlargement continued for about 12 days, at which time the tumors had reached their maximum size. Responses in the floral tube were much less striking than those of the nectary, and there was no evidence of meristematic activity in the stylar canal tissue. Divisions in other than the transverse plane were observed in the neck, particularly in the outer ring of bundles and adjacent parenchyma. Within the bundles cambial activity was initiated and a few parenchymatous derivatives

were formed. In no case were root primordia formed, and no new bundles were differentiated in the proliferated tissue. Meristematic activity ceased after a relatively short period. Thereafter all cells except mature conducting elements of the xylem increased in size, and density of protoplasmic contents decreased throughout. In old tumors the walls of eight or ten peripheral cell layers in the neck region increased considerably in thickness and showed conspicuous pits. Conducting elements of the phloem were no longer recognizable as such, and a few very thick-walled pitted fibers were sometimes found in the outer part of the external phloem.

When the transverse cut was made in such a way that only a portion of the nectary was removed, there was very little activity beneath the uninjured surface; when the cut was above the nectary, proliferation was marked only in the stylar tissues. In this case the style became considerably elongated.

Discussion

Although relatively few species have been investigated, wide variation has been found in the extent of seed-coat development in artificially produced parthenocarpic fruits. In the present investigation there was a progressive decrease from the normal seed-coat size in response to different treatments in the following order: after pollination, the seeds were large; untreated fruits and controls treated with pure lanolin produced slightly smaller abortive seeds; following indoleacetic acid at full bloom development was still further retarded; and the acid applied 4 days before full bloom resulted in the smallest seeds (approximately half the usual size). The small seed coats were only partially hardened. Fruits of the last-mentioned series were decidedly smaller than those which were pollinated. Untreated fruits and controls treated with pure lanolin were as large as those which were pollinated. In the cucumber variety studied, therefore, the presence or absence of an embryo is not a major factor affecting the size of the fruit. Whether the indoleacetic acid has a direct retarding effect on fruit development or acts through changes in the food supply or in some other manner is not known. Chemical analyses of fruits produced by artificial parthenocarpy are needed. Further histological studies to show the relative importance of cell division and cell enlargement to final fruit size would also be desirable.

Following pollination, the nectary, floral tube, and neck of the flower rapidly dry out. The development of an active growing tissue in response to early application of the acid-lanolin mixture from a portion of the flower that would soon shrivel and die in the usual course of events is of interest. Since no true abscission layer is formed at the base of the cucumber neck, this is of different character from the effects of growth substances on abscission of petioles and other organs. In contrast to the results in the bean, the proliferation of tissues of the cucumber fruit is limited to a fairly short period of time, and no vascular derivatives or root primordia are formed.

Summary

1. The development of cucumber fruits of a naturally parthenocarpic variety (Rollison's Telegraph) was studied before and following pollination. The development of megagametophyte, embryo, and seed coats was observed in detail. Tissues of the perianth, stigma, nectary, and neck shriveled within a week after pollination, and a protective periderm formed at the apex of the live tissue

beneath. Twenty days after full bloom hardened seed coats contained large well-developed embryos.

2. Development of untreated fruits in which there was no pollination was essentially the same, except for the absence of embryos and endosperm, the disintegration of megagametophytes, and the slightly smaller size of the seed coats.

3. In other cases transverse cuts were made through the lower portion of the floral tube and a 2 per cent mixture of indoleacetic acid in lanolin was applied to the cut surface.

4. When application of the mixture was made 4 days before full bloom, apical tumors were formed, involving tissues of the nectary, floral tube, and neck. These reached maximum size in about 12 days. Neither vascular tissues nor root primordia were differentiated in the tissues of the tumors. Ovules developed to about half normal size, and seed coats became partially hardened.

5. When pure lanolin was applied 4 days before full bloom, death of the apical tissue was greatly retarded and usually no periderm was formed. Ovules developed as in untreated controls.

6. When indoleacetic acid in lanolin was applied at full bloom the apical tissues remained alive but proliferated very little. Ovules were intermediate in size between those of controls and those of fruits given the pre-bloom treatments with indoleacetic acid in lanolin.

7. When pure lanolin was applied at full bloom, development was essentially the same as that of untreated controls.

8. In the unfertilized ovules of parthenocarpic fruits the nucellus generally developed into a massive tissue with a large central cavity.

9. Meiosis and megagametophyte development were not noticeably affected by any of the treatments.

10. In a single instance following premeiotic treatment with indoleacetic acid there was some proliferation of gametophytic tissue.

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EFFECTIVENESS OF GROWTH SUBSTANCES IN DELAYING ABSCISSION OF COLEUS PETIOLES

F. E. GARDNER[1] AND W. C. COOPER[2]

Introduction

An important practical attribute of the plant-hormone chemicals as a class is their ability, in varying degrees of effectiveness, to prevent or delay abscission of flowers, fruits, and leaves. It is recognized that abscission, from an anatomical standpoint, is different in various plant organs, and this fact may influence greatly the response to hormone application. The immediate object of the following experiments was to determine the relative effectiveness of a number of plant hormones in delaying leaf abscission when these chemicals were applied in various ways. In the course of the experiments some responses of physiological interest, incidental to the tests for comparative effectiveness of the compounds, were encountered and are included.

METHODS.—It is a common observation that when leaf blades are removed, leaving all or part of the petioles attached to the stem, the petioles abscise usually within a few days. LA RUE (4), using *Coleus*, demonstrated that indoleacetic acid in a lanolin paste applied to the cut ends of the petioles delayed their abscission. Basically this same procedure has been adopted in these experiments, using the time required for abscission as a measure of the effectiveness of any hormone or concentration of hormone. *Coleus*, the test plant used, has a number of advantages. Any given strain can be easily and quickly increased by vegetative means, and large numbers of uniform, single-stemmed plants can be selected for test purposes. When the leaf blades are removed the untreated petioles begin to abscise after about 48 hours, and the process is largely completed within 3 days, provided the temperature is fairly high. Hormone-treated petioles, on the other hand, may persist for weeks.

For the purpose of these experiments, uniform cuttings were taken from a "mother" bed and rooted in a greenhouse bench. The plants developed rapidly in the bench, and when approximately 8 inches tall, the petioles of the top two pairs of fully expanded leaves of each plant were used. Petioles of immature leaves were found to persist too long—even without hormone treatment—to be used for accurate test purposes. For each hormone treatment twelve plants involving forty-eight petioles were employed. In many cases twenty-four instead of twelve plants were used and the treatments replicated, so that half the plants occurred at two different locations in the bench, in order to disclose any positional effects. Although considerable differences in growth were evident in various portions of the bench, no very marked differences in time of petiole abscission within a given treatment were disclosed.

Two methods of applying each of the hormones were used. In the first method the hormones in a 5 per cent ethyl alcohol solution[3] were sprayed on the intact

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[3] Because of the relatively high concentrations of growth substances used, alcohol up to 5 per cent in the aqueous solution was employed to increase solubility. A 5 per cent alcohol solution was in itself without visible effect on the plants.

plants, and 2 days later the leaf blades were cut from the selected petioles. Abscission records were dated from the day the blades were removed. In the second method the blades were cut off with a sharp knife, and a drop of the hormone-lanolin mixture, liquefied by warming, was applied to cover the cut surface of each petiole. In this procedure two lengths of petiole, 0.5 cm. and 2 cm., were allowed to remain on the plant. Some interesting responses, involving transport of effect, were disclosed by using these different petiole lengths. Figure 1 shows individual plants with four leaf blades removed, leaving the petioles 0.5 and 2 cm., respectively, ready for application of the growth substance.

Rate of abscission, as well as effectiveness of the compounds, is unquestionably affected by temperature. With relatively low temperatures the time of normal abscission is delayed and also the effectiveness of the compounds reduced. In these experiments no special efforts were made to control the temperature of the greenhouse, but all comparable treatments were included within the same series and so were subject to the same fluctuations of temperature, light, and humidity. When one series, or bench of plants, was completed it was discarded and new plants prepared for the following series. Throughout the experiments the same strain of *Coleus* was employed.

Counts of petioles remaining on the plants after treatment were made daily or every other day, depending on the abscission rate. In making these counts the petioles were lightly touched to dislodge any which might still be attached although abscission had taken place. The various compounds were tested at four concentrations, 0.01, 0.02, 0.05, and 0.1 per cent, both as water-alcohol sprays and in lanolin. The following compounds were included in careful comparative tests:

Indole compounds	Naphthalene compounds
β-Indoleacetic acid	α-Naphthaleneacetic acid
γ-Indolebutyric acid	β-Naphthoxyacetic acid
Methyl indolebutyrate	α-Naphthylmethylacetate
β-Indole acetamide	α-Naphthyl acetamide
	α-Naphthyl thioacetamide

In addition to these recognized plant-growth substances, a large number of

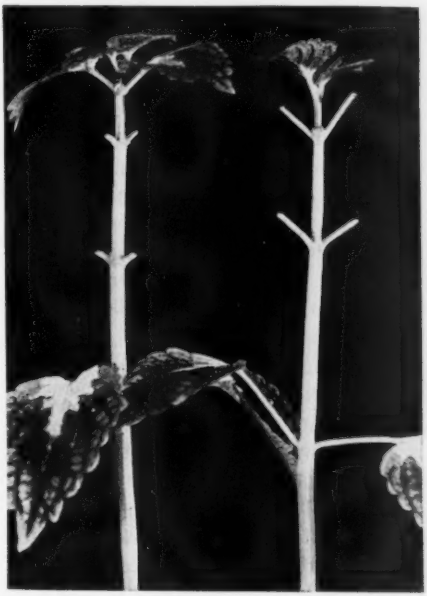


Fig. 1.—*Coleus* plants with petioles cut to 0.5 and 2 cm., ready for application of lanolin-growth substance mixtures.

miscellaneous compounds were tested for activity in delaying abscission by applying them in lanolin at 1 per cent concentration to the petiole stubs.

In preliminary tests it was found that the effect of naphthalene acetonitrile and of the propionic and butyric acids of naphthalene was so slight in delaying petiole drop that they were omitted from

further comparative tests. Likewise no effect could be demonstrated from α-naphthoxyacetic acid, although the β derivative was quite potent.

Results

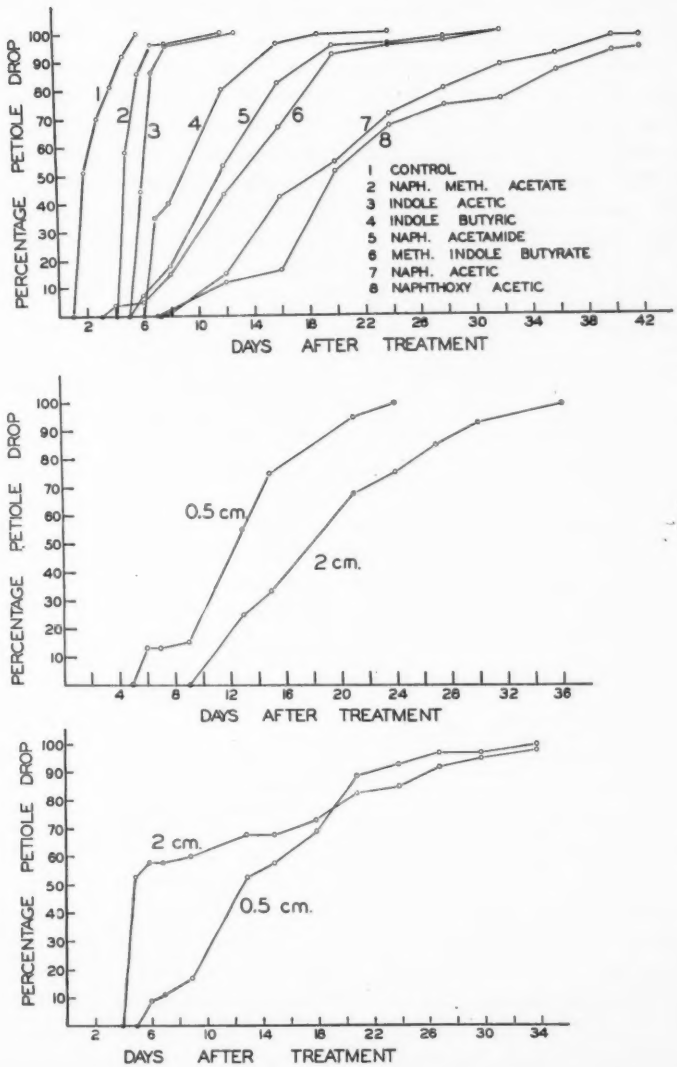
SPRAY APPLICATIONS.—At 0.01 per cent concentration most of the compounds had but slight effect in delaying abscission. β-naphthoxyacetic acid stood out pre-eminently more effective at this concentration than any other compound tested. The maximum effectiveness of all compounds was reached at 0.05 per cent, with the exception of β-indoleacetic acid, which showed still greater effectiveness at 0.1 per cent.

The increased effectiveness of all compounds, with the single exception of indoleacetic acid, with increasing concentrations up to 0.05 per cent but no greater effect at 0.1 per cent was very striking. The limitation of effectiveness of so many compounds at 0.05 per cent suggests that the limiting factor lies within the plant itself, restricting the utilization of more than a definite quantity of hormone. It is not known why indoleacetic acid, the only compound of the lot known to occur in plant tissue, was not similarly restricted, although the fact seems to be without particular significance at present.

Inasmuch as 0.05 per cent represented the approximate maximum effective concentration of each of the compounds, with the exception of indoleacetic acid already noted, the effect of the various compounds on abscission at 0.05 per cent is shown in figure 2 and represents their maximum relative effectiveness. Omitted from figure 2, to avoid crowding the chart, are the results with (*a*) α-naphthyl thioacetamide, which were nearly identical with naphthyl acetamide itself, and (*b*) indole acetamide, which showed practically no delay in abscission over the control, even when used at 1 per cent spray concentration. The comparison in figure 2 shows β-naphthoxyacetic acid to be the most effective compound tested, followed in order by α-naphthaleneacetic acid and methyl indolebutyrate. It will be noted presently that the order of relative effectiveness was quite different when the same compounds were applied by the lanolin method.

LANOLIN APPLICATIONS.—Before attempting to present the evidence on the relative effectiveness of the various compounds in lanolin applications, the factors which complicate this presentation should first be considered. The chief was the finding that the length of petiole through which the hormone must act to affect the point of abscission in large measure determined the effectiveness of a given compound. This in itself would not be any particular complication were it not for the fact that the effect of all indole compounds was increased by acting through long petioles, whereas the effect of all naphthalene compounds was thereby apparently decreased, owing to a lag in time before the naphthalene compounds became effective. Figures 3 and 4 show the abscission rates with a typical compound of each class with petiole lengths at 0.5 and 2 cm.

The time lag before naphthyl thioacetamide took effect in the case of the 2-cm. petioles permitted more than 50 per cent of the petioles to fall (fig. 4). After starting to react, however, this compound appeared to be more effective on 2-cm. petioles, as evidenced by the slope of the curve, than in the case of those 0.5 cm. long. The conclusion is probably justified that increased petiole length enhances the actual effectiveness of both indole and naphthalene compounds, but



FIGS. 2–4.—Fig. 2 (top), comparative effectiveness of various growth substances applied as sprays at 0.05 per cent concentration, in delaying petiole abscission. Fig. 3 (center), delay of abscission with indoleacetic acid, applied in lanolin at 0.1 per cent concentration, showing greater effectiveness by acting through the longer petioles; this was typical of all indole compounds. Fig. 4 (bottom), delay of abscission with naphthalene thioacetamide, applied in lanolin at 0.1 per cent concentration, showing lag in start of effectiveness with the longer petioles; this characterized the action of all naphthalene compounds.

that, owing to the lag in the case of the naphthalene compounds, increased length of petiole decreased the practical effectiveness of this latter group. The lag in the case of naphthyl thioacetamide was somewhat more pronounced than with most of the other naphthalene compounds and was therefore purposely selected to demonstrate the point.

tween control petioles 2 cm. long and those 0.5 cm. long.

It is apparent from table 1 that, as in the case of the spray applications, the 0.05 per cent mixture in general marked the maximum effective concentration. There were minor exceptions to this rule, one of which was indoleacetic acid, which has consistently showed increased activ-

TABLE 1

RELATIVE EFFECTIVENESS OF VARIOUS HORMONES IN PREVENTING ABSCISSION WHEN APPLIED IN LANOLIN TO CUT PETIOLE ENDS

Compound	Days required for 50 per cent of petioles to drop					
	0.1% conc.		0.05% conc.		0.01% conc.	
	0.5 cm.	2 cm.	0.5 cm.	2 cm.	0.5 cm.	2 cm.
Indole group						
Methyl indolebutyrate	24	29	22	25	15	18
Indolebutyric acid*	14	16	14	16	10	12
Indoleacetic acid	13	18	11	15	10	12
Indole acetamide	11	13	11	13	5	9
Naphthalene group						
α-Naphthaleneacetic acid	24	12	19	13	5	5
β-Naphthoxyacetic acid	13	10	13	8	12	9
α-Naphthylmethylacetate	11	10	11	9	9	4
α-Naphthyl acetamide	12	11	12	9	3	2
α-Naphthyl thioacetamide	11	5	11	4	2	2

*In a subsequent test the 0.1 per cent concentration of indolebutyric showed marked increased effectiveness over the 0.05 per cent. The temperature was unusually high during this latter test but is not offered as an explanation of the seeming inconsistency. It is probable that the relative effectiveness of this compound is higher than here indicated.

Any comparison of the relative practical effectiveness of the various indole and naphthalene compounds tested by the lanolin method would obviously have to take into consideration the matter of petiole length. Table 1 shows the time in days required for 50 per cent of the petioles to abscise for each of the compounds used at three different concentrations on petioles of 0.5 and 2 cm. lengths. With the pure lanolin control plants, more than 50 per cent of the petioles dropped in 2 days, and no significant difference in time of dropping could be detected be-

ity above the 0.05 per cent concentration. All the indole compounds showed greater activity with 2-cm. petioles than with those 0.5 cm. The reverse situation was true of the naphthalene compounds, reflecting the situation already described with respect to the time lag when naphthalene compounds were used on the longer petioles.

Considering all three concentrations (table 1), it is believed that the order of the compounds as given in the table represents their relative effectiveness within the indole and naphthalene groups. This

order, of course, applies to lanolin applications of the hormones to the cut ends of the petioles and differs from the order shown in figure 2 for spray applications of the same compounds.

MISCELLANEOUS COMPOUNDS.—Since a great number of miscellaneous organic compounds and a simple test method in the *Coleus* technique were available, it was decided to make at least a preliminary test of the entire group of chemicals for activity in delaying abscission. In many instances there were reasons, based on molecular configuration, for anticipating that the compounds did not possess hormone activity, but they were nevertheless employed. All the following compounds were tested at 1 per cent concentration in lanolin applied to 1-cm. petiole stubs, as previously described. Even at this relatively high concentration most of the compounds failed to produce any effect on abscission of the *Coleus* petioles. A few produced such slight effect that it would require more careful testing to establish it definitely, and even then the degree of such activity would be so slight as to preclude any practical advantage from their use. The list is presented only as a matter of record.

COMPOUNDS TESTED

1. Acetoacetanilide
2. Acetoacet-2-chloroanilide
3. Acetoacet-2, 5-dichloroanilide
4. Acetophenone
5. Acetyl-o-aminobenzoic acid
6. Acetyl-o-anisidine
7. Acetyl-p-anisidine
8. Acetyl diphenylamine
9. Acetyl-o-phenetidine
10. Acetyl-p-phenetidine
11. Acetyl phenylhydrazine
12. Acridone
13. 1-Aminoanthraquinone
14. 2-Aminoanthraquinone
15. 2-Amino-5-azoanisole
16. p-Aminoazobenzene
17. 2-Amino-5-azotoluene
18. p-Aminobenzophenone
19. p-Aminodiphenyl
20. 2-Amino-4-nitrophenol
21. o-Aminophenol
22. p-tert.-Amylphenol
23. Anethole
24. Aniline
25. Anisaldehyde
26. Anisic acid
27. p-Anisidine
28. Anisalacetone
29. Anthracene
30. Anthragallol
31. Anthranilic acid
32. Methylanthranilic acid
33. Methyl anthranilate
34. Azoxybenzene
35. Barbituric acid
36. Benzalacetone
37. p-Benzalaminophenol
38. Benzalaniline
39. Benzaldehyde phenylhydrazone
40. Benzamide
41. Benzanilide
42. Benzenoazo-o-cresol
43. Benzene hexachloride
44. Benzeneazoresorcinol
45. Benzil
46. Benzilic acid
47. Benzoic anhydride
48. Benzoin
49. Benzoyl-o-toluidine
50. Benzylaniline
51. o-Benzylphenol
52. p-Benzylphenol
53. Benzylphenylnitrosoamine
54. Benzyl sulfide
55. β'-Butoxy-β-ethoxyethyl acetate
56. p-Bromoacetanilide
57. p-Bromoanisole
58. o-Bromoanisole
59. Bromobenzene
60. o-Bromobenzoic acid
61. m-Bromobenzoic acid
62. p-Bromobenzoic acid
63. p-Bromodiphenyl
64. p-Bromophenetole
65. o-Bromophenetole
66. n-Butylbenzene
67. sec.-Butylbenzene
68. tert.-Butylbenzene
69. n-Butyranilide
70. n-Caproanilide
71. Carbazole
72. Carvone
73. Chloranil
74. p-Chloroacetanilide
75. β-Chloroanthraquinone
76. o-Chlorobenzoic acid
77. p-Chlorobenzoic acid
78. o-Chlorodiphenyl
79. Chlorohydroquinone
80. p-Chlorophenetole

81. o-Chlorophenol
82. 2-Chloro-o-phenylphenol
83. 4-Chloro-o-phenylphenol
84. p-Chlorothymol
85. m-Chlorotoluene
86. Chrysene
87. Cinchonidine
88. Cinchonidine salicylate
89. Cinnamic acid
90. Cresidine
91. m-Cresol
92. p-Cresol
93. m-Cresyl benzoate
94. p-Cresyl benzoate
95. p-Cyclohexylanisole
96. o-Cyclohexylphenol
97. p-Cyclohexylphenol
98. Dextrose
99. Diacetyl benzidine
100. 2, 4-Diaminodiphenylamine
101. 2, 4-Diaminotoluene
102. Dianisalacetone
103. Dianisidine
104. Dibenzalacetone
105. p-Dibenzoidioxin
106. Dibenzylaniline
107. p-Dibromobenzene
108. 2, 5-Dichloroaniline
109. p, p′-Dichloroazoxybenzene
110. 4, 4′, Dichloro-2, 2′-dinitrodiphenyl
111. 4, 4′, Dichloro-2, 2′-dinitrodiphenyl sulfide
112. 4, 4′-Dichlorodiphenyl
113. 4, 6-Dibromo-o-cresol
114. 2, 6-Dichloro-4-nitroaniline
115. 1, 5-Dihydroxyanthraquinone
116. 2, 4-Dihydroxyazobenzene-4′-sulfonic acid
117. 1, 3-Dihydroxy-4-chlorobenzene
118. Ethylene glycol diacetate
119. Furoic acid
120. Methyl furoate
121. n-Propyl furoate
122. n-Butyl furoate
123. n-Isoamyl furoate
124. Furfural
125. Furfuryl alcohol
126. Furfuryl acetate
127. Furfuryl diacetate
128. Hydrofuramide
129. α-Methyl-α-phenylhydrazine
130. Salicylacetic acid
131. Sulfanilic acid
132. Phenothiazine
133. N-Phenylanthranilic acid
134. Phenylacetic acid
135. Potassium-n-butylxanthate
136. Uric acid

Naphthalene compounds

137. 2, 4-Dibromo-1-naphthol
138. 2, 4-Dichloro-1-naphthol
139. α-Naphthyl acetate
140. β-Naphthyl acetate
141. β-Naphthyl benzoate
142. β-Naphthoylacetonitrile
143. Sodium-1-naphthol-4-sulfonate
144. Naphthalic acid
145. 2-Aceto-1-naphthol
146. Acenaphthene
147. α-Naphthylcarbinol
148. Acenaphthoyl-3-propionic acid
149. 1-Methyl naphthyl-4-methyl thiocyanate
150. 1-Methyl naphthyl-4-methyl isothiocyanate
151. 2-Methyl naphthyl-1-methyl thiocyanate
152. 2-Methyl naphthyl-1-methyl isothiocyanate
153. Tetralyl-6-methyl thiocyanate
154. Tetralyl-6-methyl isothiocyanate
155. α-Naphthyl methyl thiocyanate
156. α-Naphthyl methyl isothiocyanate

Discussion

In trying to anticipate which of the available growth substances to apply to correct any given abscission problem, and also what range of concentrations to employ, a number of factors should be considered. Perhaps the first of these is an anatomical consideration of the type of abscission involved. At least two general types are recognized: (*a*) that characterized by the formation of a definite pre-formed abscission layer resulting from secondary cell division such as usually precedes the dropping of flowers, leaves, and young fruits; and (*b*) that characterized chiefly by weakening changes in the cell walls in an abscission zone, as has been reported by MacDaniels (**5**) and McCown (**6**) preceding the drop of mature apple fruits. Delay of abscission by growth substances in one instance retards cell division, whereas in the other instance cell-wall weakening may be interrupted. The distinction between the two types of abscission is not a hard and fast one, for in the type characterized chiefly by cell-wall changes some cell division is also found, and in the first type cell-wall changes in the newly formed abscission layer subsequently take place before actual abscission occurs. Myers (**7**), in a study of abscission

in *Coleus* leaves, described these changes. He also reported that the application of growth substances to debladed *Coleus* petioles retarded the initiation of the abscission layer and its growth at any stage of development. The growth substance most effective in one type of abscission is not necessarily most effective in the other. Unfortunately, evidence is not available on a sufficient number of plant species to support a statement as to which growth substances are most effective in delaying each type of abscission. For example, the abundant evidence on the effect of the various growth substances in the rooting of cuttings shows that the different species and varieties differ considerably in their relative response to the various substances. As to range of concentrations to be employed, the limited evidence available suggests that the low concentrations, in the neighborhood of 0.0005 per cent, found to be very effective against abscission of the second type, as in the preharvest drop of apples (**2**), are much too dilute to be of value in the first type, of which leaf abscission is an example.

Another factor, although probably over-emphasized in the consideration of the choice of growth substance for a particular purpose, is its relative solubility in the carrier to be used. For practical purposes it is probable that a relatively low solubility, within reasonable limits, resulting in an incomplete solution, does not necessarily impair the effectiveness of a compound but may, in fact, enhance it by providing a film of finely divided particles on the surface of the plant which may gain entrance over an extended period. The excellent results obtained by HOFFMAN (**3**) in controlling apple drop by hormone dusts are evidence of this, as are also the results with lanolin-emulsion sprays for various purposes and the use of hormone dusts for rooting of cuttings. It should be recorded that in these tests it was not possible with the spray applications to effect complete solution of all the compounds at the higher concentrations, even with 5 per cent alcohol which was used as a carrier. All compounds were in complete solution at the 0.01 per cent concentration, but at 0.05 and 0.1 per cent, methyl indolebutyrate, naphthyl methylacetate, naphthyl acetamide, and thioacetamide were incompletely dissolved. As suspensions or emulsions they were delivered to the plant surface in the same absolute amounts as the other compounds completely in solution. The lanolin mixtures also may not have been complete solutions, for, even though heated and the compounds apparently dissolved in the melted lanolin, it was not possible to be sure that, when cooled, the solubility was not exceeded. It is, of course, not possible to say whether the relative results with the various compounds would have been different had they all been completely dissolved. For the practical considerations involved it makes little difference, inasmuch as the results of any comparative tests of these compounds must of necessity be qualified by the solubility of the compounds and by many other unknown characteristics, such as the relative rate of entrance into the plant tissue, their rate of movement in the tissue, and their relative inactivation or destruction by enzymes or other compounds within the plant.

Entirely apart from its effectiveness for controlling abscission, another factor to be considered in the selection of a growth substance is the avoidance of certain undesirable deforming effects. β-naphthoxyacetic, indolebutyric, and α-naphthaleneacetic acids are especially prone to result in aerial rooting and

marked distortion by bending and twisting of young foliage and shoots. Certain "formative effects" with β-naphthoxyacetic acid and certain other compounds have been noted (**10, 11**). In general, these undesirable side effects are severe only on immature or succulent growth and with relatively high concentrations of the compounds. On the other hand, naphthalene acetamide is practically free of these effects.

Two interesting features have been disclosed in this study which may eventually prove important in helping to understand the nature of the hormone action in preventing abscission and in the practical application of these substances to counteract undesirable dropping of plant organs. These two features are the delay in effect of the naphthalene compounds when applied to the petiole some distance from the point of abscission and the increased effectiveness of the indole compounds—and probably also of the naphthalene compounds, once their action begins—by acting through the longer lengths of petiole.

The time lag in the action of the naphthalene compounds through long petioles can probably be ascribed to a slower rate of longitudinal transport of this group of compounds than is characteristic of the indole compounds. No suggestion is advanced as to why this should be true. WENT and WHITE (**9**) present evidence which indicates that naphthaleneacetic acid is transported longitudinally through *Avena* coleoptiles at a much slower rate than either of two indole compounds also tested. From the standpoint of practical application of the naphthalene compounds this slower rate of transport has significance only when the growth substance must be applied some distance from the point of desired activity. With short petioles, where the distance between point of application and point of abscission is small, or with spray applications in which the compound reaches the abscission point directly, the lag was obviated.

The increased effectiveness by acting through the longer petioles in the case of the indole compounds, and of the naphthalene compounds as well—except for their lag—suggests that the action of the growth substances depends on or is conditioned by the presence of a necessary second substance or substances already present in the plant tissue. Obviously there should be more of the second substance or substances in a long petiole than in a short one, and the amount present may be a factor limiting the action of the growth substance itself. The maximum effective concentration of so many of the growth substances in this study at approximately 0.05 per cent lends further support to the presence of naturally occurring factors within the plant which limit the effectiveness of applied growth substances. It is not known, however, whether the naturally occurring factor or factors are simply metabolic substances such as carbohydrates and proteins, which are undoubtedly essential to all activity by the plant and have been shown by various workers to influence the response to applied growth substances, or whether these factors can be classified as specific hormone-like substances designated as "calines" by WENT (**8**). EYSTER (**1**) has recently proposed that the action of "auxins" is due fundamentally to the release of diastase and possibly other enzymes from the protein colloids to which they are normally attached.

Summary

1. The relative effectiveness of a number of growth substances, both indole and

naphthalene derivatives, in delaying leaf abscission in *Coleus* was tested by two methods of applying the compounds: by aqueous sprays and by lanolin mixtures applied by hand to cut petiole surfaces.

2. In the case of the spray applications the maximum effectiveness in delaying abscission was reached at approximately 0.05 per cent concentration of all compounds, with the exception of indoleacetic acid, which showed still greater effect at 0.1 per cent concentration. In most instances with the lanolin applications 0.05 per cent also represented the approximate maximum effective concentration.

3. The relative order of effectiveness of the various compounds differed considerably in the two methods of application. At the maximum effective concentration methyl indolebutyrate was the most effective in lanolin applications on petiole stubs, whereas in the spray application method β-naphthoxyacetic acid proved most effective.

4. With all napthalene compounds applied by the lanolin method there was an appreciable lag between time of application and the beginning of effect in preventing abscission, while the indole compounds took effect promptly. The results suggested that the naphthalene compounds were transmitted longitudinally through the petioles at a slower rate than the indole compounds.

5. The indole compounds, and also the naphthalene compounds, once their action was initiated, exhibited greater effectiveness on petioles 2 cm. long than on 0.5 cm. lengths. This suggested the possibility of the presence in the petioles of a naturally occurring second substance or substances necessary for the action of the applied growth substance, although it does not imply that such substances are specific hormone-like factors.

6. The prevention of leaf abscission appears to require a much higher concentration of applied growth substance than the prevention of mature fruit drop. This may be related to the differences in the types of abscission involved.

7. Of 156 additional miscellaneous organic compounds tested for activity in delaying petiole drop, none showed sufficient activity for practical usage.

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DEVELOPMENT OF THE FIBROUS NET IN THE FRUIT OF VARIOUS RACES OF LUFFA CYLINDRICA

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Introduction

The fibrous network of the fruit of *Luffa cylindrica*, the "vegetable sponge" or "sponge gourd," is of considerable economic importance, since it is used for a wide variety of purposes, from bath sponges to oil filters in marine engines. The literature on these plants does not include any account of the development and histology of the fibrous net or a comparison of its character in the various types. This paper presents the results of such a developmental analysis on a number of diverse races in this species.

Seeds of *Luffa* were obtained from various commercial sources and from the Department of Agriculture; they originated from several warm-temperate regions, notably Japan, Afghanistan, and various parts of the United States. About thirty distinct races were grown. All were planted outdoors in the Marsh Botanical Garden at New Haven, Connecticut, early in May, 1942. Some of the most promising lines were also started in the greenhouse and set out in the field when danger from frost was past. These for some time maintained their size advantage over the ones planted outdoors. Many of the races grew poorly and were obviously unsuited to our climate, but a considerable number successfully produced mature fruits. It is only these latter which were intensively studied.

In addition to *L. cylindrica*, which is the chief source of the commercial product, a number of races of *L. acutangula* were also grown. Only a brief report of these will be made in the present paper.

METHODS.—The plants, well fertilized, were grown on a trellis and were planted 9 feet apart each way. No serious trouble from insect or fungous pests was encountered nor were any of the plants affected by mosaic. The season was an unusually good one, and there was no killing frost until November.

The races of *L. cylindrica* chiefly studied included several of the long narrow type, with a length of about 1200 cm. and a width of 70 cm.; others somewhat shorter and wider (600 × 100 cm.); and others considerably smaller (fig. 1). For the various types, self pollinations were successfully made and abundant seed obtained.

By the use of various substances, especially indoleacetic and naphthaleneacetic acids, parthenocarpic fruits were produced. This was accomplished most readily by applying the substance in lanolin paste directly to the stigma on the day preceding the opening of the flowers.

Polyploid plants were produced by treatment of seedlings with a 0.5 per cent solution of colchicine, but they failed to grow vigorously and none produced fruit.

Ovaries were collected at all stages, from a diameter of 1 mm. or less in width to mature fruit. In larger ovaries samples from various regions were taken. Material was killed in Craf, run through butyl-alcohol, imbedded in paraffin, and stained with safranin and fast green. In later stages of net formation, entire young fruits or parts of them were dissected and studied directly by hand sec-

tions and by maceration. In each race, entire mature fruits were harvested and dried and the leathery outer pericarp removed, exposing the entire network. By removing the seeds and retting in water, the fibrous net, free from other structures, was isolated.

tire strand with Ruthenium red and studying under low power.

Results

The ovary is divided into four rather well-marked regions (fig. 2). The central, ovule-bearing portion may be termed the placental region (*D*). The morphological nature of the rest of the ovary is not well understood, but for convenience it may be termed the "wall." Running through it is a series of large vascular bundles, setting off the outer wall (*A*) from the inner portion. In the latter, two definite regions may be distinguished, the inner wall in which the strands of the fiber run

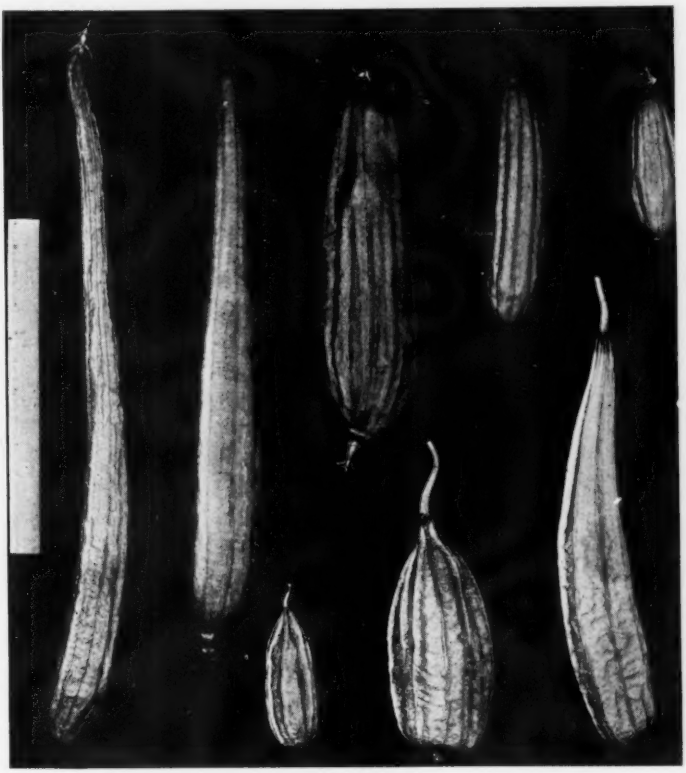


FIG. 1.—*Luffa* fruits: upper row, *L. cylindrica;* lower row, *L. acutangula*

Maceration of the mature strands into their component fiber cells proved difficult but was accomplished by prolonged boiling in concentrated potassium hydroxide. Details of the fibers could be brought out well by staining in Ruthenium red or safranin. The relationship of the fibers on the surface of the strand could be determined by staining the en-

in a chiefly longitudinal direction (*C*) and the middle wall where they run parallel to the transverse circumference (*B*). In the very smallest ovaries the provascular cells of the main vascular bundles, running lengthwise, begin to appear; but when the ovary reaches a diameter of about 1 mm., differentiation of the fibrous network begins. This occurs both in the placental region and in the wall by

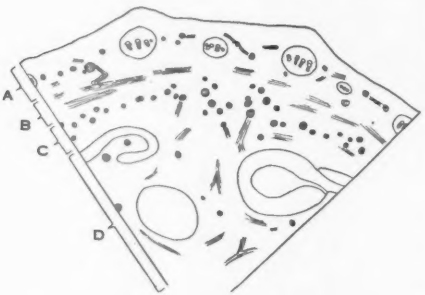


FIG. 2.—Diagram of cross sector of young ovary of *Luffa*. *A*, outer wall showing main longitudinal vascular bundles and small anastomosing fiber strands; *B*, middle wall showing outer transverse strands of fibrous network (outer shell separates from net along line *AB*); *C*, inner wall showing longitudinal fiber strands; *D*, placental region showing ovules and anastomosing fiber strands.

a change in the character of the otherwise uniform parenchymatous cells.

The first visible indication of the future position of a strand is the increased protoplasmic content of a continuous series of parenchyma cells (fig. 5). Each of these soon begins to divide parallel to the axis of the strand. The strand is laid down as a unit simultaneously throughout its course and differentiation does not proceed from cell to cell. Whatever determines the pattern of the future net seems to affect the entire structure and modifies the physiological condition of a series of cells connected with one another in a closed system.

The entire net is not laid down at once, but new strands continue to be differentiated from essentially isodiametric parenchyma cells until some time after fertilization, and often until the fruit is nearly half grown. Thus there is a continuous increase in the complexity of the net pattern as the fruit increases in size. Bundles in the inner wall appear somewhat later than the rest, for this part of the wall develops later. The cessation of strand differentiation in a given region directly follows the cessation of cell division in the parenchymatous cells of the ground tissue in this region. *Luffa* generally agrees with the other cucurbits (5) in which cell division ceases first in the placental region, next in the inner wall, and then in the outer wall. Thus the differentiation of new strands ceases first in the central portion of the fruit and successively in the outer regions.

The strands of the net are not distributed at random but form a very definite pattern which is fundamentally alike in all types but shows certain specific differences in the various races. Around each ovule or group of ovules there is a series of strands, and the mature seed is thus held in a fibrous pocket with an opening on its axial side through which the seed ultimately falls and then drops out of a pore at the tip as the dried fruit is shaken. Surrounding the placental region in the innermost portion of the wall is a series of strands developing comparatively late, often stouter than the rest, most of which run parallel or nearly parallel to the long dimension of the fruit. Outside these is another series of strands which tend to run at right angles to the axis and thus transversely around the outside of the net. In the more delicate fruits there may be only one layer of strands in each set, but in the larger ones there may be many strands and a much more massive system. No bundles pass

upward from the stalk directly into this net, but the vascular supply of the net is entirely through connection with the main vascular bundles in the outer wall, which are continuous with the bundles of the stalk and stem.

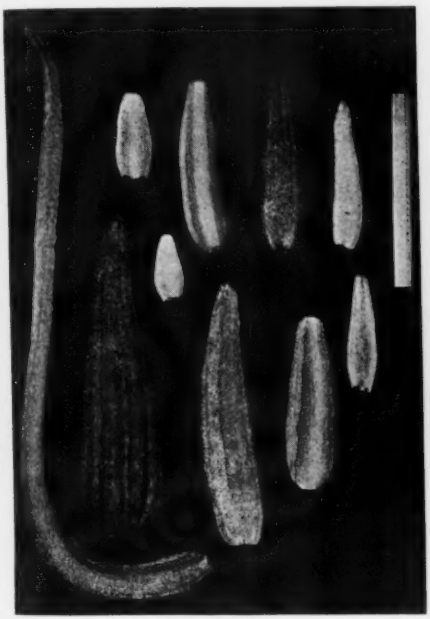


FIG. 3 —Fibrous nets of some of the races studied, showing differences in color, form, and texture.

No strand of the net extends for more than a few millimeters without branching, so that the strands anastomose freely and the entire fibrous system is a closely intercommunicating reticulum. Its details vary considerably in the different races. Some of the more important differences, both as to the whole net and as to its details, are shown in figures 3 and 4.

At maturity the outer wall, most of the cells of which are now sclerotic, becomes a tough dry shell which separates readily from the fibrous net within. This shell contains the main vascular bundles, which are continuous with the vascular system of the stem. Connecting with them and ramifying through this outer wall are many small strands which are continuous with those of the inner net.

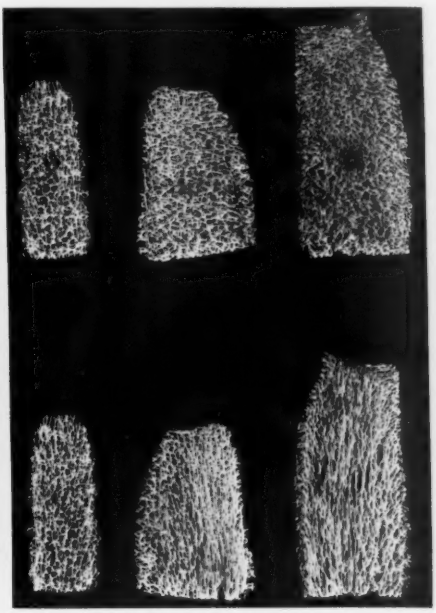
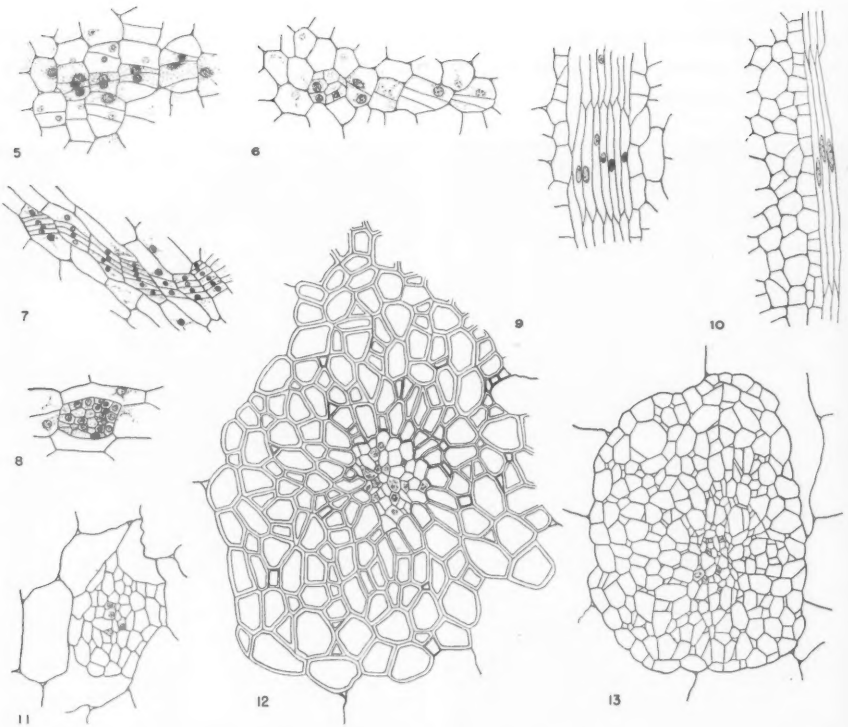


FIG. 4.—Pieces of wall of net from corresponding regions in three races. Above, as seen from fruit surface; below, as seen from inside. Outer series of bundles prevailingly transverse in direction, the inner prevailingly longitudinal. Races differ markedly in radial thickness of fiber system, compactness of network, and diameter of strands.

When the mature wall separates from the net these connections are broken.

In *L. acutangula* there is no sharp line between the sclerotic and the thin-walled parenchyma in the outer layers of the fruit, the former gradually merging into the latter. It is therefore impossible to separate easily the tough outer wall from the fibrous net within, so that this species is much less satisfactory for the production of "sponges" than is *L. cylindrica*.



FIGS. 5–13.—Fig. 5, early stage in formation of a transverse fiber strand, from transverse section through ovary 1.5 mm. wide. ×200. Fig. 6, early stage of strand formation showing (at left) cross section of strand mother cell divided into nine cells and (at right) longisection of young branch strand. ×200. Fig. 7, longisection through slightly older transverse strand showing fiber initials in ground parenchyma cells. Note absence of relation between course of strand and shape of mother cells. ×200. Fig. 8, transection through inner longitudinal strand, somewhat older than that in fig. 6. ×200. Fig. 9, more advanced stage of fiber development with end walls pointed; ovary diameter 10 mm. Note group of fibers which have come from same mother cell. ×200. Fig. 10, still more advanced stage of fiber development showing fibers from only one side of strand; ovary diameter 26 mm. Single group of fibers can still be distinguished. ×115. Fig. 11, transection through young fiber strand of small size with phloem elements in center, from placental region in Line A_3. Ovary diameter 8 mm. ×225. Fig. 12, transection through comparable mature fiber strand in same region and line as in fig. 11. Ovary diameter 75 mm. ×225. Fig. 13, transection through fully grown but not yet lignified fiber strand of medium size, showing phloem elements in center and numerous small lumina with acute wall angles, presumably resulting from intrusive growth and branching of fibers. Line D_2. Ovary diameter 110 mm. ×115.

The histology of the individual fiber strands varies considerably in different parts of the fruit and among different races. In the rows of densely protoplasmic cells which are the first indication of the position of a strand, the cells begin to divide parallel to the axis of the future strand (figs. 5, 6). This direction bears no necessary relation to the particular shape of the cell, since the strand may twist and turn rather irregularly through the mass of parenchyma. Each cell is cut by these divisions into a considerable number of small elongate cells, the whole group retaining for a time the size and shape of the mother cell (fig. 7). If cut transversely, this group is seen to consist of about 10–20 cells (fig. 8). The young strand at this stage is thus a series of such cell groups, each derived from a single mother cell.

As the fruit expands, these elongate cells grow in length though but little in width. The surrounding parenchyma cells continue to divide. Thus series of long narrow cells, connecting with others to form the young strands, may now be seen in the midst of isodiametric parenchyma cells. For considerable time the groups of cells which were formed from a single mother cell may be distinguished, since they are of the same length (figs. 9, 10). Their end walls, however, instead of being transverse are now becoming pointed, and many of the cells begin to show some resemblance to fibers. During this period longitudinal divisions may occur, so that the number of cells in a strand increases somewhat (fig. 11). Some transverse divisions are also found. The parenchyma cells immediately adjacent to the fiber strand divide more frequently than the others, so that a sheath of relatively small cells is differentiated around the strand. In some cases there is evidence that these cells may actually add to the size of the strand.

Relatively early in development some internal differentiation also arises in the strand itself. Several of the innermost cells begin to take on the character of phloem elements. In the larger bundles one or more ringed or spiral vessels make their appearance.

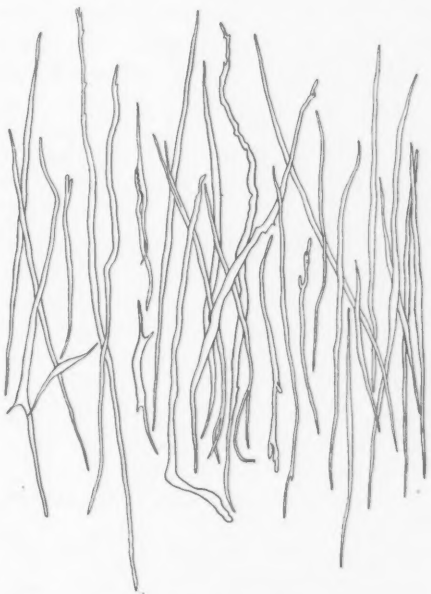


FIG. 14.—Typical fibers isolated by maceration from outer transverse strand in Line A5. Ovary diameter 100 mm. Both straight and forked or branched forms are shown, some of which are short elements from strand periphery, forming transitions to sclerified parenchyma cells. Longest fibers are about 3600 μ long and 55 μ wide. ×24.

In a typical fibrous strand almost all the cells ultimately become thick-walled, elongate fibers (figs. 12, 14). Their growth in length at first keeps step exactly with the increase in size of the fruit itself. This may be shown by measuring the length of typical young fiber initials

from comparable strands and plotting them against the width (or length) of the ovary in which they occur. After these initials reach a length of about 200 μ, it becomes evident that they are growing in length faster than the dimensions of the fruit. Measurements of the fibers in the maturing fruit are not easy to obtain, since an entire cell can rarely be observed in a single section, and since they are still too delicate to macerate. As they cease growing, however, these fibers develop thick and heavily lignified walls and can be separated by maceration. Measurements at this time show them to have reached a much greater length than could be accounted for by expansion at the same rate as the fruit. Thus in a certain race fiber initials from the outer wall were on the average 30 μ long in ovaries 2.5 mm. wide, 60 μ in those 6 mm. wide (about the time of flowering), and 250 μ in those 25 mm. wide. Fibers from these same strands at maturity were 2500 μ long in fruits 100 mm. wide, whereas they would have been only about 1000 μ long if they had continued to increase at their earlier rate, corresponding to that of the fruit. This must mean that the developing fibers continue to elongate after the fruit has slowed down or stopped and that their ends grow past each other intrusively (**6**). This conclusion is also supported by the fact that the number of fibers seen in cross section of a mature strand is considerably greater (twice or more) than the number in a young strand (fig. 12 as compared with fig. 11).

Thus intrusion evidently takes place before the heavy lignified wall is formed. That it occurs chiefly at the ends of the fibers is suggested by the fact that at maturity the ends are very much thinner than the middle regions. The ends are also frequently forked, as though elongation had been blocked. The whole fiber is often gnarled and irregular in shape, with projecting portions at various points, as though the whole wall at one time had been more or less plastic and had grown into any cavity adjacent to it. These irregular shapes occur most frequently in those parts of a strand which branch or are sharply bent. Here also the fiber ends are often twisted around each other (fig. 14). Much of the increase transversely in cell number of the strands is probably due to the presence of these branched ends of the fibers. The very acute angles of the fiber walls, as seen in cross section, are quite different from those of normal tissue and further suggest that many of these cells have reached their present position by intrusion (fig. 13).

Strands in the same region may differ considerably in the length of their fibers. Those with shorter fibers are presumably the ones which differentiated later from ground parenchyma, and in many cases this can be shown to be true. Thus the small strands in the outer wall and those arising as secondary anastomoses in the inner region, both of which appear relatively late, have the shortest fibers of all.

During their development the diameter of the fiber elements increases somewhat. At their first differentiation they are approximately 3 μ wide and at maturity 25–50 μ wide. The stouter strands tend to have somewhat wider cells, though the chief factor in strand diameter is the number of its cells.

The thickness of the fiber walls does not vary considerably in various parts of the fibrous net. Conspicuous thickening of the wall does not occur until the fibers cease growing. The walls are often markedly thinner near the ends of the fibers, indicating that thickening began later here than in the middle. Lignification does not proceed at the same time every-

where but occurs in some strands before others and in some portions of a strand earlier than in others. In all parts of the net the fibers were so tightly cemented together that only a drastic maceration technique was able to separate them.

The general character of the net and its constituent fibers seems to be essentially the same in parthenocarpic fruits as in those with seeds, although in the former the fruit and its net are usually somewhat smaller.

In the very elongate fruits pollination was often not complete, with the result that only the apical portion of the fruit produced seeds and grew to its normal diameter. The basal portion was relatively slender, but its fibrous skeleton, although relatively small, was found to resemble that of the fertilized portion. Its strands were markedly narrower and its fibrous cells smaller, however. Development was evidently arrested and maturity was reached before normal size had been attained.

The various races studied differ somewhat in the character of their fibers. In most of them, such as A5 and B10, the fibers reach a length of 3500–3800 μ (average 2400 μ), but in a few cases—such as B2—no fibers longer than 1900 μ were found, and many were shorter. This is a small-fruited line with a rather delicate net. Its fibers could also be macerated with greater ease than in the other lines. In general there tends to be a relation between small fruit size, delicate fibrous net, and shorter fibers. Degree of lignification does not seem to be related to fruit size.

Discussion

The development of the fibrous net of *Luffa* is a good example of the way in which a bundle system may become differentiated in the midst of relatively undifferentiated parenchymatous tissue. This may often be observed in other plants in the origin of the veins and fiber strands in leaves and fruits. Sometimes differentiation begins very early, going back almost to the start of growth. In other cases, as in that of the fibrous net here described, it may not begin until the general form of the organ and the demarcation of its various regions have been attained. The differentiation of the net bears little visible relation to this earlier differentiation, the strands twisting and branching among the orderly rows of the parenchyma cells in the young ovary primordium without relation to the direction of these rows or the shape of their cells. It is almost as though one pattern had been superposed on another.

In such cases, relatively large and often highly vacuolate parenchyma cells, if they happen to lie in the course of one of these developing strands, will become meristematic again and divide actively although their neighboring cells go on to maturity undivided. These illustrate the fact recently emphasized (7) that division in embryonic tissue is by no means limited to small, richly protoplasmic cells but often takes place in large and vacuolate ones. This differentiation also recalls the development of strands of xylem and phloem in parenchymatous tissue through regeneration, as described by FREUNDLICH (1) and others, and the origin of vascular connections between adventitious buds and deeply seated bundles, as shown by various writers.

Other members of the Cucurbitaceae show similar development of a vascular net, but in most of the genera—such as the various squashes, melons, and cucumbers—the strands consist only of phloem and parenchyma, the latter of relatively long but unlignified cells. These strands are most conspicuous in

the coarse and "stringy" pumpkins but are present even in fruits with the most delicate flesh.

Continued elongation of fibers after their adjacent cells have stopped growing is not very common. TAMMES (8), MEEUSE (4), and MAJUMDAR (3), working with fibers and collenchyma of various sorts, have found that the elongation of most of these cells does no more than keep pace with the elongation of the organ of which they are a part. On the other hand, many cases are known where sclereids of various sorts elongate enormously, growing in between the cells of adjacent tissues for relatively great distances. VAN TIEGHEM (9) has described such cells in the leaves of Peneaceae and JADIN (2) in those of Simarubaceae, and numerous other cases are known. BLOCH has recently studied their development in the air roots of *Monstera*. Some of the greater length of such pointed cells may be accounted for by differential wall growth, by means of which their ends become progressively more acute. In such extreme cases as the present one, however, it must be due to the continuance of growth by one cell after its neighbors have stopped growing or are elongating in an opposite direction. Such growth seems to be of the type called intrusive by SINNOTT and BLOCH (6), where one region of a cell, usually its tip, actually grows in between others, much as does a tylosis, rather than by gliding over the surface of adjacent cells. That growth of the *Luffa* fibers is chiefly at their ends is indicated by the much thinner walls there. The two halves of a pit are always opposite each other, showing that there is no gliding after the pits are formed. Critical evidence from other sources that cells do not glide makes it unlikely that these fibers and sclereids are exceptional in their behavior.

The intrusive growth of *Luffa* fibers appears to be associated with their specialized character, since in the corresponding strands of other cucurbits, where the cells remain thin walled these never seem to attain greater length than could be accounted for by growth of the entire organ.

The morphological nature of the *Luffa* strands is also a matter of some interest. There are transitions of various sorts from the typical fibrovascular bundles of the outer wall to the typical strands of the fiber net. The latter seem to be vascular strands in which the xylem and phloem have been much reduced (the xylem often wholly absent) and in which the fibrous tissue is greatly developed. Their conductive function, which presumably was primitive, has here largely been replaced by a mechanical one involved in the support and dispersal of the seeds.

No sharp line can be drawn between the typical vascular cells in these strands and those which belong to the surrounding ground tissue. Cells transitional in shape and pitting between xylem cells and fibers are not uncommon. Even more frequent are cells intermediate between typical fibers, which presumably are "vascular" in origin, and the relatively short sclerenchymatous cells of the ground tissue. Such transitions are common in the outer wall, where most of the ground tissue is sclerified. They may also be seen at the surface of many of the strands, where the cells of the sheath, intermediate in shape between parenchyma and fibers, often have thick lignified walls. Such cases demonstrate the impossibility of drawing sharp lines between categories of tissues, such as "vascular" and "fundamental." The character of each cell seems to be determined by the position it occupies in a developmen-

tal pattern rather than by its membership in a particular lineage or category of cells.

The usefulness of the *Luffa* fiber depends in part on the fact that its strands form a continuous and closed network. The diameter of the strands and their number per unit volume make this network relatively coarse or fine. The durability of its fibers depends upon the degree of lignification of their walls and on the strength with which the cells are bound together by the intercellular substance. All these traits, as well as the size and shape of the net, show considerable genetic variability and may be made the basis for experiments in breeding and selection. They could doubtless be modified considerably by this means, and types of sponges could be developed which would have many more economic uses than *Luffa* does at present.

Summary

1. The development of the fibrous network is described for a number of races of *Luffa cylindrica*, the "vegetable sponge."

2. The strands begin to differentiate when the ovary primordium is about 1 mm. wide, and new ones continue to form until cell division ceases. A complex anastomosing network is produced.

3. The first indication of the appearance of a new strand is the increase in protoplasmic content of a row of parenchyma cells. These then divide parallel to the axis of the future strand but with no necessary relation to the shape of the mother cells or to the pattern of the ground tissue.

4. The cells thus formed develop into the fibers. They elongate as the surrounding parenchyma grows but continue to grow intrusively, chiefly at their tips, for some time after fruit growth ceases.

5. Most strands consist chiefly of fibers, but there are a few phloem elements in the middle of each and often one or more xylem cells. The strand is a modified vascular bundle.

6. Fibers range in length from very short ones to about 3800 μ, being on the average 1500–2000 μ long. Many are branched or have irregular shapes. They are very difficult to separate by maceration.

7. The various races differ considerably in the development of the fibrous net, in the diameter and spacing of its strands, and in the length and character of the fibers.

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EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND DEVELOPMENT OF KOK-SAGHYZ

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Introduction

The influence of photoperiod on the growth and development of *Taraxacum kok-saghyz* Rodin has been determined to provide information that may be of use in establishing the production of this crop on an economical basis in this country. The length of the photoperiods and the temperature at which they are applied are important factors in the production of many crops, since they limit the range in which the plants can be grown, influence the time of flowering, control the vegetative development of the plant, and affect its chemical composition.

Since little was then known concerning the photoperiodic and other environmental requirements of kok-saghyz, the first shipment of seed from U.S.S.R. was distributed by the Rubber Plant Investigations Project for testing in certain localities of the United States and Canada where photoperiodic and other environmental conditions were similar to those prevailing in that part of central Asia where the plant is native(1). In addition to these studies under natural environment, investigations of the effects of photoperiod and of the interaction of photoperiod and temperature on the growth and development of this plant were conducted at Beltsville by the Photoperiodic Project.[4] Knowledge of the nature and extent of the response of kok-saghyz to these factors should be useful in the selection of the most promising localities in which to establish this crop and may suggest methods of culture which will speed the development of new types and may aid in the selection of those progenies best adapted to a particular region.

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Experimental results

Plants for these studies were secured from field plantings in various states and from seedlings grown in the greenhouse. All the seedlings were started in flats and transplanted when they had two or three leaves. They grew well under the various experimental conditions and comparatively few were lost.

GREENHOUSE EXPERIMENTS.—Photoperiods of 8, 10, 12, 14, 16, and 18 hours were maintained. The natural photoperiod was extended with Mazda light of about 50 foot-candles, and the individual lots of plants were covered with a double layer of black sateen at the close of their respective photoperiods. These covers were removed at 8:00 A.M. the following morning.

The first greenhouse experiment was concerned with the influence of photoperiod on the development of flower buds that had been previously initiated on plants growing in the field.

A supply of plants was obtained during January, 1943, from St. Paul, Minnesota; Parma, Idaho; Klamath Falls, Oregon; Berkeley, California; and from Beltsville, Maryland. Represent-

ative plants from each source were examined with a wide-field binocular microscope, and flower primordia were found to be present on almost every plant. These primordia were in the very earliest recognizable stages of development on some plants but had developed to a size easily visible with the naked eye on others.

Plants from each locality were potted in 6-inch pots and subjected to the six photoperiods being maintained. Ten to twenty plants from each locality were placed on each photoperiod. The plants quickly resumed growth and within a few weeks all of them flowered. The lots on 8-and 10-hour photoperiods were slightly later in blooming than the others and formed somewhat shorter flower stalks, but these differences were not sufficiently great to be considered significant.

In this experiment relatively little seed was formed on any of the plants that flowered unless the flowers were artificially cross-pollinated. Since seed was formed abundantly on all lots following hand cross-pollination, it seems evident that the photoperiods were in no way responsible for the lack of seed production. WARMKE (**2**) has found that there is a high degree of self-sterility in kok-saghyz and that in screened greenhouses, or when insects are scarce, lack of cross-pollination is the main cause of low seed setting.

The results of this experiment indicate that growth and development of flower buds initiated in the field before the treatments were started were not inhibited by any of the photoperiods employed. The range in length of these photoperiods was about as great as would be encountered in the field in any part of the United States during the growing season. So far as photoperiod alone is concerned, it would therefore appear feasible to move plants from one section of the country to another for seed-production purposes if it were desirable to do so.

Further greenhouse experiments were concerned with the influence of photoperiod on the initiation of flower primordia in seedlings. The seedlings were started in January, 1943, from seed obtained from the Rubber Investigations Project of the Bureau of Plant Industry, Soils, and Agricultural Engineering. The seed was secured from Russia through the offices of the State Department.

From the time of planting the seedlings were maintained on daily photoperiods of 8, 10, 12, 14, 16, and 18 hours. As soon as the seedlings had developed two or three small leaves they were transplanted to 2-inch pots, in which they were grown until they had produced rosettes slightly larger than the diameter of the pot. The plants were then shifted to 4-inch pots. The experiment was replicated three times. Replicates 1 and 2 were in a single compartment of the greenhouse and replicate 3 was in an adjoining compartment. During most of the experiment the temperature of the latter compartment was maintained about 5° F. warmer than that of the former.

All the plants made satisfactory growth, but those receiving photoperiods of 12 hours or more developed slightly larger rosettes and appeared somewhat more vigorous than those on the two short photoperiods. The leaves of the plants receiving long photoperiods grew semi-erect, in contrast to the prostrate leaves of the plants on short photoperiods.

Dissections were made of two plants from each photoperiod on February 27, when the plants were about 6 weeks old, to determine the earliest time at

which flower primordia were initiated. The stem terminals of all these plants were forming vegetative leaves, and no flower primordia were present. A week later microscopic flower buds were present on two of twelve plants dissected. Dissections were discontinued at this time because data concerning subsequent development of the plants were desired. Further data on floral initiation were based on the time of appearance of macroscopic buds.

These buds were visible in various stages of development on many plants on March 20. Flower buds appeared on 174 plants, or 33 per cent of the plants of the experiment, during the period March 20 to May 8, and about two-thirds of these were present before April 1. Their formation had nearly ceased by May 8, only five plants being recorded during the week immediately preceding this date. The percentages of plants forming flower buds on photoperiods of 8, 10, 12, 14, 16, and 18 hours were 15, 24, 36, 37, 48, and 37, respectively. The percentages for replicates 1, 2, and 3 were 42, 38, and 20. Variance analysis of the data indicates that the differences between the photoperiods and also between the replicates were significant at the 5 per cent level but not at the 1 per cent point.

Flowering was not completely inhibited by any of the photoperiods, although the data suggest that the 8- and 10-hour photoperiods were somewhat less favorable than the four longer ones.

The low percentage obtained in replicate 3 may have resulted from the somewhat higher temperature of that section of the greenhouse. Further data relating to the influence of temperature will be presented later.

During February a second lot of seed was planted for a repetition of the preceding experiment. In this second greenhouse experiment the same photoperiods were tested as in the previous one. The experiment was set up in duplicate instead of triplicate, with the two replications in the same greenhouse compartment. Two soil types, one a composted topsoil from Beltsville and the other a highly alkaline one from Oregon, on which a very satisfactory crop of kok-saghyz had been grown the previous season, were employed. The pH of the Beltsville soil was 5.8 and that of the Oregon soil 9.05. The number of plants per sub-lot was 50, or a total of 1200 in the entire experiment.

All the seedlings grew very well, but those in the Beltsville soil were considerably more vigorous than those in the western soil. As in the previous experiment, larger, more erect growth was again observed in the plants subjected to longer photoperiods. Only 43 of the 1200 plants of the experiment formed visible flower buds before June 1, and all but four of these were plants growing in Beltsville soil. Only one plant of those receiving 8-hour photoperiods and only six of those on 10-hour photoperiods formed flower buds. The remaining 36 plants were about equally divided among the four longer photoperiods.

This experiment was started about a month later than the preceding one, and the higher temperatures then prevailing may have been responsible for the very much lower percentage of plants forming flower buds.

Results of the two experiments indicate that floral initiation occurs in seedlings subjected to any of the photoperiods tested but is somewhat more abundant on plants receiving photoperiods of 12 hours or more.

GROWTH-CHAMBER EXPERIMENTS.—Two experiments were conducted in

growth chambers concurrently with the experiments in the greenhouse. In these a study was made of the interaction of temperature and photoperiod on growth and development of the plant.

For the first experiment the temperatures of the four available chambers were adjusted to 55°, 65°, 75°, and 85° F., respectively. The chambers were illuminated with a combination of carbon arc and Mazda light. The intensity of the Mazda light in all chambers at the plant surface was about 160 foot-candles; that of the arc light in chambers 1 and 2 was about 1200; and that in chambers 3 and 4 about 1800.

Photoperiods of 10, 13, and 16 hours were obtained in each chamber by covering certain plants with lightproof covers at appropriate times. The operation of the Mazda and arc lights was controlled by means of electric time-switches set to turn the lights on at 8:00 A.M. and off at midnight. The 10-hour plants were covered at 6:00 P.M. and the 13-hour ones at 9:00 P.M.

Seedlings were started in the greenhouse on photoperiods of 10, 13, and 16 hours, respectively. As soon as they had two or three leaves they were transplanted to 3-inch pots filled with composted soil and transferred to the growth chambers. The plants were held in these chambers from February 5 to March 8, 1943. The experiment was discontinued on March 8, following a failure of mechanical equipment in the 65° room which resulted in loss of the 10- and 13-hour plants and in slight damage to the 16-hour ones. All the plants at this time were moved into a greenhouse, where they received photoperiods of 16 hours or more daily until May 6.

Pairs of plants from each temperature and photoperiod were photographed on March 4, after 29 days in the growth chambers (fig. 1). Considerable variability existed within each lot, and the plants photographed were selected to represent as nearly as possible the average size for the lot.

The largest plants in each chamber were associated with the longest photoperiod. This difference between plants of different photoperiods was most prominent in the 55° and 65° chambers and least in the 75°. The intensity of radiation in each room was of course constant throughout the period of illumination, so that the total amount of radiant energy received by plants of different photoperiods in any room was in the same relationship as the daily duration of light received by them. Since the size of the plants on the different photoperiods is thus correlated with duration of illumination and with total amount of radiation, the differences between them may have resulted primarily from either of these factors.

The plants produced in the 75° and 85° chambers were characterized by a prostrate habit and gray-green color, while those of the two lower temperatures were of a yellow-green color and their leaves were semi-erect. These differences in color and growth habit may have resulted from differences either in temperature or in light intensity, because the rooms at the two higher temperatures were equipped with arc lights of considerably higher intensity than those of the other two rooms. The differences, whatever their cause, persisted long after the treatments at different temperatures and photoperiods were discontinued and were still evident when the plants were finally discarded on May 6.

Although the plants did not flower abundantly, differences associated with both temperature and photoperiod were

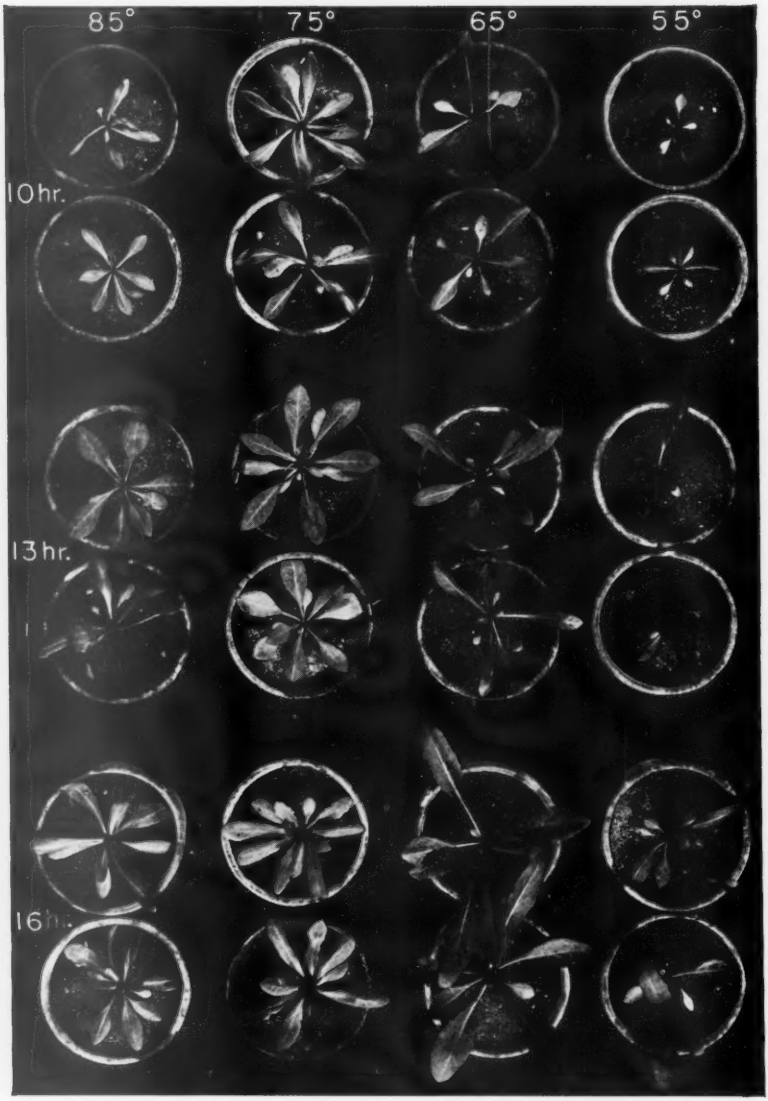

FIG. 1.—Kok-saghyz seedlings after 29 days in growth chambers at temperatures indicated and at daily photoperiods shown. Only two or three small leaves present on seedlings at beginning of experiment.

apparent. In the 55°, 75°, and 85° lots, flowers were formed by six, eight, and twenty-nine plants, respectively, and at photoperiods of 10, 13, and 16 hours on seven, six, and thirty plants, respectively. The greatest number of flowers in any one treatment was in the 55° lot that received 16-hour photoperiod. Although the 65° plants held on 16-hour photoperiod were continued in the greenhouse, no data are presented from them because they had been damaged before their removal to the greenhouse.

The results of this experiment suggest that low temperature and long photoperiod are favorable to the initiation of flower buds by kok-saghyz seedlings.

The second experiment in the growth chambers was started on March 10 and continued until April 27. In this experiment 12- and 16-hour photoperiods were maintained at temperatures of 65° and 75°F., and the plants were grown in two different types of soil under each of these conditions. The soil types were a composted topsoil from Beltsville and an alkaline soil from Oregon. This experiment was performed in duplicate since two chambers were available for each temperature. The temperatures of the various chambers were so arranged that each replicate included one with a high-intensity arc light and one with a low one. This was done to determine whether or not the prostrate habit of the plants grown in rooms 3 and 4 of the previous experiment resulted mainly from the higher temperature or from other characteristics of those chambers.

In each chamber the plants were divided into four main groups of eighty plants, forty in Beltsville soil and forty in Oregon soil. Two of the groups received 12-hour photoperiods and the other two 16-hour.

Growth of plants under all the experimental conditions was very good, but the plants in the Beltsville soil were somewhat larger than those in the Oregon soil. The prostrate habit was again prominent in chambers 3 and 4. Since one of these chambers was operated at 65° in this experiment it appears probable that the prostrate habit of growth observed in the former experiment was in some way correlated with conditions in the chambers other than temperature.

While the plants were still in the growth chambers, forty-two of them formed flower buds and a few blossomed. These forty-two plants were distributed among the various treatments as follows: thirty-three were in Beltsville soil and nine in Oregon soil; thirty-five received 16-hour photoperiod and seven received 12-hour; sixteen were at 65° and twenty-six at 75°F.

In this experiment the plants remained in the growth chambers 18 days longer than in the previous one. Formation of flower buds was not observed in the first experiment while the plants were in the chambers, but in the second experiment flower buds were visible on many of the plants before they were removed. In the first experiment flowering was greater in the lots that had received the lower temperatures, while in the second the reverse effect was observed. The data in the former were obtained from plants a few weeks after the treatments with photoperiod and temperature had been discontinued and in the latter while the treatments were still in progress. The apparent reversal disappeared, however, after the plants were removed to the field. Further data on this point are presented in the next section.

OUTDOOR PLANTINGS.—Plants from certain of the experiments in the green-

house and growth chambers were transplanted outside, so that any effects of treatment that appeared later in their development might be observed. Three such plantings were made, one from the second growth-chamber experiment and two from greenhouse experiments.

The plants of the second growth-chamber experiment that had not produced visible buds on April 27, the date on which the treatments with temperature and photoperiod were discontinued, were transplanted to the field.

By June 4 a total of seventy-nine plants had developed visible buds or had flowered in the field. Sixty-seven of these were from the chambers that had been operated at 65° and twelve were from the 75° chambers. By July 3, fifty-three additional plants from the low temperature chambers and thirty-three from the higher temperature chambers had formed buds, making a total of 119 and forty-five, respectively. Apparently some effect of the lower of the two temperature treatments applied in the growth chambers was expressed in the later development of the plants and resulted in the formation of flower buds on a greater number of them. No such effect resulting from photoperiodic treatment was observed.

The plants for the second outdoor experiment were grown in the greenhouse from March 10 to April 17 under the six conditions of photoperiod of the greenhouse experiments. During this time a few of the seedlings bloomed, but toward the end of the period blooming had nearly ceased and most of the plants had discontinued expanding new leaves. Since this condition did not occur in the growth-chamber experiments, it was thought that possibly the high temperatures in the greenhouse during the daytime inhibited their development. Consequently on April 17 most of these plants were transferred to the growth chambers, where part of them were held at 65° and the rest at 75° until May 4. A few plants from each photoperiod were kept in the greenhouse as controls. On May 4 all the lots, totaling 960 plants, were transplanted to the field. During the next 2 months only nineteen plants of this entire planting formed flower buds. Most of the plants grew very little during this 2-month period. Occasional plants developed vigorous rosettes but failed to form flower buds, even though the plants were considerably larger than others that blossomed. The few plants that flowered were not restricted to any particular treatment.

Another lot of plants that had received approximately the same treatment in the greenhouse as those of the second outdoor experiment constituted the material for the third outdoor experiment.

These seedling plants were started in the greenhouse in 2-inch pots in early February on six different photoperiods replicated three times. They were grown in these pots for approximately 2 months during which time a few plants in some of the lots formed buds or bloomed. On April 6 all the lots were moved into another greenhouse, where they received the natural photoperiods then prevailing. On April 10, twenty plants from every lot, or a total of sixty from each of the six photoperiods, were transplanted outdoors into boxes of soil $1 \times 1 \times 3\frac{1}{2}$ feet in size and filled with well-composted soil. Ten plants were transplanted to each box. On April 20 a similar lot of plants was also transplanted.

The 10-day interval between the two plantings was much colder than the 10-day period following the second planting. The maximum temperature during

this first period was 64°, and on two days it was 42° and 43°, respectively. Minimum temperatures were below freezing on three nights and were less than 10° above freezing on five other nights. During the 10-day period from April 20 to 29, the maximum temperature was 79° and the lowest maximum any day was 55°. No freezing nights occurred, and on only six nights was the temperature less than 10° above freezing. Minimum temperatures after April 29 were nearly all above 50° F. Both lots thus received low temperatures after they were transplanted, but the first lot received considerably more cold than the second.

On May 27, 47 days after the first transplanting, flower buds or flowers were visible on 88 per cent of the first lot and on 59 per cent of the second. On June 25 these percentages had risen to 98 and 88, respectively. These results suggest that possibly the low temperatures received shortly after transplanting may have stimulated the formation of flowers. No differences in time or percentage of bloom were correlated with any of the treatments received prior to transplanting.

Summary

1. Flower primordia were present on plants of *Taraxacum kok-saghyz* collected from several localities in the central or northern parts of the United States during the late fall or winter months. Photoperiods of 8, 10, 12, 14, 16, and 18 hours did not inhibit the development of these primordia into mature flowers when the plants were transplanted in the greenhouses.

2. Wide variability of leaf pattern was observed in the seedlings, but morphological characteristics of the flowers indicated that almost all were kok-saghyz plants. Seedling plants subjected to six different lengths of photoperiods varying from 8 to 18 hours flowered under all conditions, but more of them flowered with photoperiods of 12 hours or longer than with shorter photoperiods.

3. Plants that received low temperatures during their early development flowered more abundantly than those that received no low temperature. Ninety-eight per cent of one lot of seedlings that had received several weeks of cold weather during the spring flowered, while less than 2 per cent flowered in a similar lot that received no low temperature.

4. Results of these experiments indicate cool temperature and long photoperiod as conditions most favorable to early blooming of seedlings.

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SECONDARY ASSOCIATION OF FRAGMENT CHROMOSOMES IN GENERATIVE NUCLEUS OF TRADESCANTIA AND ITS BEARING ON THEIR ORIGIN[1]

C. P. SWANSON

Introduction

Many plant and animal species are known to possess fragment chromosomes in addition to their normal complement (**6**, table 16). In *Tradescantia* they are rather widespread, particularly in the *virginiana* group, and more especially in those species closely related to *T. paludosa* (**1, 4, 5, 9, 14, 17**). These fragments, in *Tradescantia*, measure about one-sixth to one-ninth the length of the major chromosomes, possess a centromere, and pass through the division cycle with little irregularity. So far as is known, they exert no genetic influence, for as ANDERSON and SAX (**1**) point out, as many as twelve can be present in a single plant with no appreciable effect. In this respect they behave much as the B-chromosomes in maize, and like them they are undoubtedly composed to a large extent of heterochromatin.

There is some disagreement as to their structure. WHITAKER (**17**) has considered them to possess a terminal centromere. In some species inversion crossing-over has revealed the fragments to be atelomitic, with the centromere nearly median (**9, 14**). Since in interspecific hybrids (for example, *T. paludosa* × *virginiana* or *T. paludosa* × *canaliculata*) the fragments pair readily (SWANSON, unpublished), this seems to be satisfactory ground for assuming that throughout the genus they are all structurally similar, recognizing, however, that the fragment can change shape and size through crossing-over (**5**). Recent findings on the instability of the truly terminal centromere (**7, 12**) also tend to support the contention that the centromere is not terminal, and additional support for this is gained from this study of the fragment in the pollen tube, where the morphology is almost diagrammatic.

The origin of the fragment chromosomes in *Tradescantia* has not been satisfactorily determined. That they are homologous, at least in part, with the major chromosomes there seems no doubt (**5, 9, 14, 17**), since pairing at meiosis has been frequently observed between them and the major chromosomes. WHITAKER (**17**), however, finds no such pairing in *T. paludosa*, the species studied here. Meiotic association has been most frequently detected at terminal regions of the major chromosomes, although lateral pairing in the median region of the arms (**4**) and centric pairing (**14**) have also been reported. It is not known whether such associations are the result of true chiasmata, such as DARLINGTON (**5**) has shown can be produced terminally. As to their origin, two tentative suggestions have been advanced. Basing his hypothesis on the fact that no terminal pairing was found at meiosis, WHITAKER (**17**) concludes that they have resulted from a fragmentation process which eliminated a major portion of both arms. On the other hand, SWANSON (**14**), finding both distal and proximal pairing at meiosis, suggests that they might have arisen from crossing-over in overlapping inversions, thus preserving both the centric and terminal regions intact while eliminating the median portions of both arms.

Secondary association provides a further means of attacking this problem. This type of loose pairing is most generally found at meiosis in organisms with small chromosomes (**3, 10, 16**), and wherever existing, it presents a means of rec-

[1] Journal article no. 627, new series, Agricultural Experiment Station, East Lansing, Michigan.

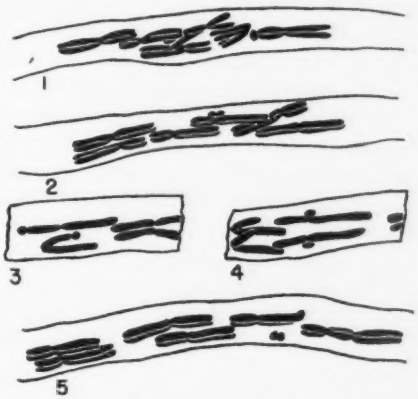
ognizing homologies. It is found less frequently in somatic cells, but a recent study of the pollen-tube chromosomes in *Tradescantia* has shown that the fragments reveal a marked tendency to associate with the major chromosomes at centric and terminal regions. This paper deals with this particular aspect of the problem and its bearing on the origin of the fragments.

A diploid ($2n = 12$) clone of *T. paludosa* Anders. & Woodson, possessing two fragment chromosomes, furnished the material for this study. The pollen tubes were grown on an agar-gelatine-sugar medium (**15**), with enough colchicine replacing the acenaphthene to give a 0.001 per cent solution. The colchicine seemed in no way to affect the secondary association but merely served to spread the major chromosomes in the pollen tube, thus facilitating the analysis. All preparations were fixed for 10 seconds in alcohol-acetic and then stained with aceto-carmine.

Observations

Since the compactness of prophase in the pollen tubes is such that it prohibits a detailed analysis, only metaphase configurations were studied to determine the pairing relationships with the major chromosomes. Each fragment was classified in one of three categories (table 1). When paired terminally, the fragment was closely attached to the end of the chromosome arm (fig. 1); when paired at the centromere, it was usually so arranged that the axes of both fragment and major chromosome were parallel (fig. 2). All other fragments were considered to be distributed at random, although it was evident that many of them were loosely associated with a particular chromosome. Those definitely classified therefore represent the minimum number showing secondary association.

A total of 800 fragments were studied, these being taken from several permanent preparations made at various times. The percentages of paired associations varied widely among the slides, being as high as 50 per cent in some instances and as low as 28 per cent in others, with an average of 42 per cent (table 1). This distribution is obviously not at random, particularly since it became clear that the fragments were associated with a particular kind of major chromosome.



FIGS. 1–5.—Fig. 1, fragment paired terminally; fig. 2, fragment paired at centromere; figs. 3, 4, separation of fragment and major chromosome with persistent secondary association; fig. 5, normal complement of chromosomes; fragment "at random."

TABLE 1

DISTRIBUTION OF FRAGMENTS IN POLLEN TUBES OF T. PALUDOSA

At random	Terminal pairing	Centric pairing	Total
463	169	168	800

The haploid complement in *T. paludosa* consists of two isobrachial and four heterobrachial chromosomes (fig. 3). Within these two groups it has not been possible to distinguish individual chromosomes, but terminal pairing of the fragment always involved the shorter arm of one of the heterobrachial chromosomes, while the same one or one similar to it was invariably associated with the fragment whenever centric pairing oc-

curred. While it is impossible to state that the pairing in each instance involved the same chromosome, a study of those nuclei possessing two fragments makes this a reasonable assumption. In no case was a nucleus with two fragments found in which both were paired terminally, although one nucleus was observed in which two fragments were paired at the centromeres of different chromosomes, both heterobrachial. This could be taken to mean that centric pairing was less specific than terminal pairing, or that two of the major chromosomes shared a region in common homologous with a portion of the fragment. No nucleus was found with one fragment paired terminally and the other at the centric regions, thus making it uncertain whether the major chromosome involved in terminal pairing is the same as that showing centric pairing.

The terminal and centric associations frequently persisted, even after the chromatids had become widely separated (figs. 4, 5); but there is no evidence for believing that the union was other than temporary or that it involved somatic crossing-over.

Discussion

DARLINGTON (3) first realized the importance of secondary association as a means of identifying homologies, and he has used this method in determining the degree and kind of polyploidy in such genera as *Prunus*. LAWRENCE (10) working with *Dahlia*, and UPCOTT (16) with *Aesculus*, have reported similar successes. In somatic cells, exclusive of dipteran salivary glands, this phenomenon has best been shown in the Diptera (11), and STURTEVANT and DOBSHANSKY (13) found, through the use of translocations, that it is dependent upon a part-for-part homology. Besides the secondary association shown in this study, the only other instance known to the writer is that in the somatic cells of *Dahlia* (10), although here the association is radial rather than parallel. A study of microspores and root-tip cells in *Tradescantia* revealed no pairing of any kind, and it seems plausible to assume that the limitations of space in the pollen tube permit the forces of attraction to function more effectively.

The pairing evidence supports the conclusions previously drawn from meiotic data (14) that the fragment is homologous with one or more of the major chromosomes and that the homologous portions are at the distal and proximal regions of the chromosome arms. More specifically, it can now be shown that one end of the fragment is homologous with a terminal region of one of the heterobrachial chromosomes, and also that another portion of the fragment, probably in the neighborhood of the centromere, is homologous with centric regions in one and possibly two of the heterobrachial chromosomes. It appears reasonable to assume that these pairings are specific as to homology. Of course there remains the possibility that these regions are heterochromatic, in both the fragment and major chromosomes, and that their pairing is of a nonspecific kind; but in view of the specificity of somatic pairing (13) and the lack of evidence pointing to a somatic pairing of heterochromatic regions, this explanation appears unlikely.

These conclusions regarding the structural affinities of the fragment are not in agreement with the conclusions of ANDERSON and SAX (1) and WHITAKER (17), who feel that the fragment represents a median segment of one of the larger chromosomes. On the other hand, it is possible that the fragments dealt with are structurally different, for as DARLINGTON (5) points out, a fragment may, through crossing-over, undergo a change in shape

and size. However, the fact that hybridization studies show the fragments in different species to be very similar, and the fact that WHITAKER studied the same species used here, indicate that the fragments are in all probability structurally alike.

Granting that the fragment is derived from a major chromosome, the diminution of chromatin can result from several processes: fragmentation, intercalary deletion, overlapping inversions, and translocation. The first can be eliminated without further consideration, since the evidence that the terminal regions of the fragment and one of the major chromosomes are homologous indicates that simple fragmentation, or loss of the distal portion of the chromosome arm, could not lead to the structure observed.

Each of the other three methods, or a combination of them, could lead to the origination of a fragment such as that found in *Tradescantia*, that is, one where the median regions of at least one arm have been eliminated while the terminal and centric portions remain intact. The data, of course, permit a discussion of the derivation of only one end and some other portion of the fragment that is homologous with the centric region of at least one major chromosome.

Intercalary deletions, one in each arm, though possible, seem unlikely as a source of fragment chromosomes, since the infrequency of spontaneous breaks and the absence of intercalary losses (8) make such an event, involving four separate breaks, of considerable rarity. A more plausible explanation can be derived from a consideration of overlapping inversions. The presence of an acentric ring chromosome in meiosis of *Tradescantia* indicates the presence of either overlapping or adjacent inversions (14). If of the former type, figure 6 illustrates the manner by which an arm of a chromosome can be shortened by the transfer of chromatin to its homologue. Crossing-over in region *B* produces greatly abbreviated and greatly lengthened arms, *ABG* and *ADCFEBCDEFG*. Arm *ABG* has the terminal and centric regions intact, and pairing could then take place between it and its progenitor chromosome. The other arm could be lost in a similar manner or in other ways.

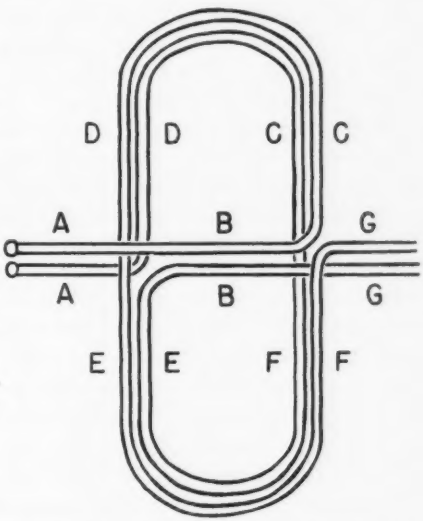


FIG. 6.—Diagrammatic representation of pairing in overlapping inversions. Cross-over in region *B* leads to greatly altered chromatids.

Reciprocal translocations suggest the simplest means of fragment production. An unequal translocation, involving breaks near the centromere of one chromosome and the distal end of another, would yield a chromosome with a subterminal centromere that would be viable, since no loss of chromatin results. This could be carried on until chance provided the opportunity for similar translocation in the other arm. If the material in the fragment were largely heterochromatin, as the genetic evidence seems to bear out, then this provides a method

not only for the origin of fragments but also for a reduction in chromosome number with no essential loss of chromatin.

Since in nuclei with two fragments both have not been seen to pair simultaneously with different chromosomes or with both ends of the same chromosome, it seems probable that while one end of the fragment possesses terminal homologies with the other chromosomes, the other end does not. The explanations then apply only to one arm, and until further data are obtained, they should be considered only in the light of suggestions.

WHITAKER (17) considers it very likely that the fragment arose in a polyploid species of *Tradescantia* because of the greater survival value aneuploids would have where the genom is duplicated. The present phylogenetic knowledge of the genus makes the reverse appear more plausible. Ample phyto-geographical and cyto-taxonomic evidence has shown that most, if not all, of the tetraploid *Tradescantia* have arisen from their diploid progenitors through autopolyploidy (1, 2), and since the diploids possess the fragment it is probable that the fragments originated in them before they gave rise to the tetraploids.

Summary

A study of 800 fragment chromosomes at metaphase in the pollen tubes of *Tradescantia* revealed that somewhat less than one-half of their number show secondary association with the major chromosomes, pairing taking place at either the ends of the chromosomes or at the centric regions. Judging from these pairing relationships, the fragment, which possesses a median centromere, has the end of one arm homologous with the distal portion of one of the heterobrachial major chromosomes, while the centric region is probably homologous with a similar region in the same or another of the heterobrachial chromosomes. The end pairing is apparently specific; the centric pairing may or may not be. The possible origin of one arm of the fragment is discussed; nothing much can be stated as to the structural makeup of the other arm of the fragment.

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PROVITAMIN A AND VITAMIN C IN THE GENUS LYCOPERSICON[1]

RALPH E. LINCOLN, F. P. ZSCHEILE, J. W. PORTER, GEORGE W. KOHLER, AND RALPH M. CALDWELL

Tomatoes are a good source of both provitamin A (β-carotene) and vitamin C, and they are of greater value in human nutrition as a source of essential vitamins and minerals than as a source of energy. Studies here reported were made to determine the genetic variability in the contents of provitamin A and vitamin C of commercial varieties and related forms and species of *Lycopersicon*.

According to MULLER (3), the genus *Lycopersicon* includes six species, five of which have been introduced into the United States by the Division of Plant Exploration and Introduction, U.S. Department of Agriculture. The species are widely divergent in fruit, vegetative, and growth characteristics, and it is reasonable to expect that considerable differences may exist also in their contents of provitamin A and vitamin C. The experiments were conducted at Lafayette during the summer of 1942. At least five plants of each strain were grown, and usually a sample of fruit was collected for analysis from a single plant that appeared typical of that strain. Samples of fruit for analysis were collected from August 18 to September 28. A control variety, Baltimore (Indiana strain), was planted every fifth row and was analyzed at intervals throughout the season. This variety served as a control on variation in vitamin content arising from environmental differences in the plot and due to different dates of sampling.

Carotenoid pigments were extracted with an acetone-hexane mixture. Carotenols (5) were removed by successive extractions with 6 per cent aqueous diacetone-alcohol, 90 per cent methanol, and methanolic KOH. Spectroscopic observations on the solution of carotenes were made for the estimation of lycopene. The β-carotene fraction was separated from the lycopene fraction on a MgO column, and measurements of absorption were made at several wave-lengths for the determination of the former pigment.

In the analysis for vitamin C, the extract was prepared according to MORELL'S procedure (2) for plant materials, and an aliquot of the metaphosphoric-acid extract was titrated with sodium 2, 6-dichlorobenzenoneindophenol (1).

The ranges of β-carotene, lycopene, and vitamin C contents, and percentage dry matter of the fruits in the various species and strains are given in table 1.

The content of β-carotene does not appear to be a distinct species characteristic, although *L. peruvianum* has a much lower average content than the other species analyzed. The content of β-carotene varied most widely in a segregating population from the cross *L. hirsutum* × *L. esculentum*, back crossed to *L. esculentum*. Since *L. hirsutum* did not fruit in the field, no carotene analyses have been made of this species, and it has been impossible to determine whether this parent is responsible for the high carotene content or whether it is due to new gene combinations. The latter possibility seems reasonable, since *L. hirsutum* has "green" fruits as does *L. peruvianum*, and the commercial parent has been found to have a low β-carotene content. The extreme variation in lycopene content may be noted in the table. Although lycopene has no known nutritional value, the red-

[1] Journal Paper no. 96 of the Purdue University Agricultural Experiment Station. This investigation was supported in part by a grant from the Nutrition Foundation, Inc.

ness of the fruit appears to be related to its lycopene content and is of interest because of consumer demand.

The commercial strains of *L. esculentum* are reasonably uniform for a low content of vitamin C as compared with either *L. pimpinellifolium* or *L. peruvianum*. Since the characters of the commercial tomato are derived almost entirely from *L. esculentum*, this is not surprising. REYNARD and KANAPAUX (4) reported a similar range in content of vitamin C in these species, except that *L. peruvianum* was found to have a range only slightly greater than that of *L. pimpinellifolium*.

The variation in genetically diverse material was much greater than that in the control variety, Baltimore, which could be ascribed to soil variability or date of sampling.

This investigation has shown that the strain highest in β-carotene contained nine times as much as the highest commercial variety. The strain highest in vitamin C contained three times as much vitamin C as the highest commercial variety. These high strains contained approximately thirteen times as much β-carotene and four times as much vitamin C as the average content of the most widely grown commercial varieties.

These strains are of little commercial value without further development but are valuable for use as parents in a program to develop high vitamin varieties of commercial tomatoes. How rapidly such varieties may be developed will depend to a great extent upon the genetic complexity of the inheritance of vitamin content. It appears unlikely that the maximum variation in vitamin content has been found.

These investigations are being continued toward the object of developing varieties of high vitamin contents.

TABLE 1

RANGES OF CAROTENE, LYCOPENE, VITAMIN C, AND DRY-MATTER CONTENTS IN LYCOPERSICON SPECIES AND CROSSES

(FRESH-WEIGHT BASIS)

SPECIES OR CROSS	NO. OF STRAINS ANALYZED FOR		β-CAROTENE FRACTION (MICROGRAMS/GM.)	LYCOPENE (MICROGRAMS/GM.)	VITAMIN C (MICROGRAMS/GM.)	DRY MATTER (%)
	Carotenoids	Vitamin C				
Red- and yellow-fruited species						
L. esculentum						
Commercial varieties	23	31	2.1–8.0	32–111	104–446	4.0–8.0
Baltimore (Indiana strain)	*	†	4.9–7.9	44–111	193–316	4.5–6.2
Foreign strains	108	161	1.0–19.1	0.8–187	143–735	4.0–13.4
L. pimpinellifolium	25	30	6.6–19.2	37–463	401–865	5.9–16.8
Green-fruited species						
L. peruvianum	18	18	0.7–3.6	None–1.4	386–1194	8.3–14.8
Cross of red-×green-fruited species						
[F_1(L. esculentum×L. hirsutum)×L. esculentum]	86	107	0.6–67.5	None–115	84–534	3.4–10.7

* Four observations. † Six observations.

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MAINTAINING THE SURFACE OF HONES

Hones that have become hollowed and scratched can be reconditioned and maintained in optimum condition by the following procedure.

Hollows and scratches are ground off with abrasive paper or cloth having particles of fine texture. Hones with relatively deep hollows can be flat-surfaced in less than 15 minutes through the use of an electric wood-sanding machine. Padding should be removed from the backstop to provide a firm flat surface against the back of the abrasive belt. When a machine is not available, the hone is rubbed against an abrasive sheet fastened to a flat surface. This method is quite satisfactory, particularly if the hone is reconditioned before the surface has become too worn.

Striations produced by the grinding can be removed by rubbing together two flat-surfaced hones. This operation can be shortened by the use of a very fine abrasive, and the grit produced during this polishing should be continuously washed away. When running water is not available, washing in still water should be frequent. Polished surfaces can be readily achieved by rubbing together two yellow Belgian hones or a yellow Belgian and a German blue-water hone; more time is required to finish the surfaces when two blue hones are rubbed together, since then there is a greater tendency toward scratches.

Hone surfaces can be maintained in optimum condition by such reconditioning after each use. The grinding operation need be repeated only when it becomes evident that polishing for a reasonable time is insufficient to keep the surfaces flat.—MARTIN HAWRYLOW, *New York State College of Forestry, Syracuse, New York.*

MORPHOLOGICAL AND CYTOLOGICAL STUDIES ON CARICA PAPAYA

LOIS THOMSON FOSTER

Carica papaya, native to tropical America, where the fruit is an important article of diet, is now cultivated extensively in all tropical regions, as well as in Texas and Florida. Papain, a digestive enzyme, is obtained from the latex of this plant. The papaya plant is a semiwoody tree which grows to a height of 10–25 feet.

Although considerable work of a physiological nature has been reported, the morphological and cytological work concerning *C. papaya* seems to have been rather incomplete, and accounts differ as to the development of the megagametophyte and embryo in this particular species. USTERI (10) reported that the megagametophyte was of the normal type, with the antipodals disappearing soon after their formation. He observed the development of an axial row but stated that the micropylar megaspore was the functional one. Although KRATZER (3) confirmed the report of a normal type of megagametophyte development, he was undecided as to the number of cells composing the axial row and claimed that either the chalazal or the micropylar megaspore might become functional. The pollen tube was described by KRATZER as a large structure which, as it enters the nucellus, becomes somewhat arched and forms small pouches. KRATZER compared the pollen tube in *C. papaya* with that found in some of the Cucurbitaceae by KIRKWOOD (2).

Contrary to the views of the earlier workers, HEILBORN (1) reported that the nucleus of the megaspore mother cell divides to form a binucleate embryo sac and that no tetrad of spores is produced, although reduction division occurs. The five-nucleate mature megagametophyte observed by him was classified as a reduced *Lilium* type or as a modification of the *Plumbagella* type. Few observations have been made concerning embryo development in this species.

SCHAFFNER (4), working with pistillate plants in the greenhouse, induced nearly mature fruits to develop without pollination through reduction of the transpirational surface of the plants.

MATERIAL AND METHODS.—The plants were grown in the greenhouse at the University of Wisconsin from seed obtained in Florida. Although both hermaphroditic flowers and dioecious types are formed, this investigation was limited to a study of pistillate and staminate plants. The fruits of these forms are characterized by a more spherical or ovoidal shape in comparison with the elongated parthenocarpic fruits.

Pistillate flowers were hand-pollinated by slightly untwisting the petals of a partially opened flower and dusting the pollen on to the stigma (9). Following pollination, measurements of the width and length of the pistils were made at intervals of 2–3 days.

A series of pistillate flowers and pistils at various stages of development before and after pollination were removed and fixed in F.A.A. or Karpechenko's modification of Navashin's solution, after being dipped into Carnoy's solution. The material was then carried through a butyl-alcohol series for dehydration and imbedded in paraffin. Sections were cut at 10–12 μ. Material was stained in safranin and fast green, in Chlorazol Black E, but best results were obtained by the use of Delafield's haematoxylin diluted to one part of haematoxylin to three parts of distilled water.

Pollen grains were germinated artificially, according to the method described

by TRAUB and O'RORK (7). The pollen was sprinkled on to slides covered with a thin layer of agar sucrose medium.

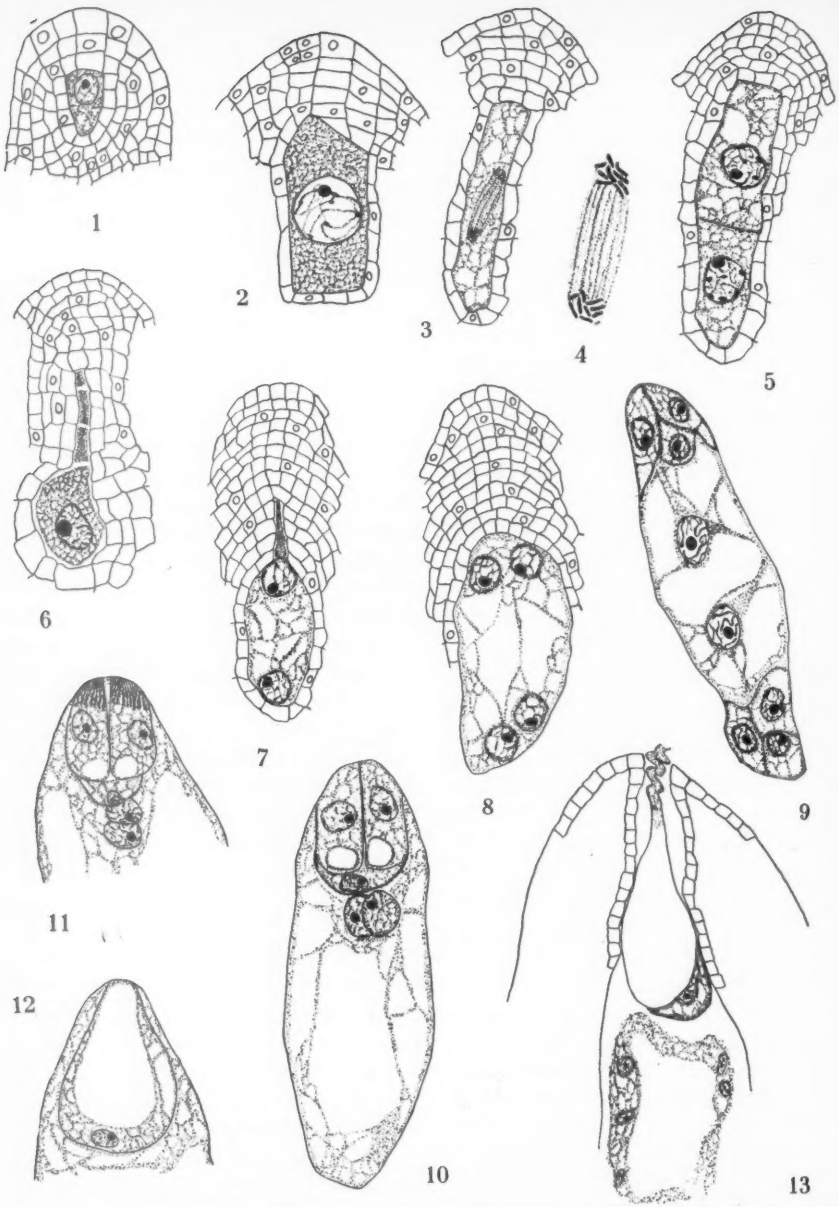
Observations

The nucellus of the young ovule appears as a slight enlargement at the end of an unusually long stalk (fig. 27), and the primordium of the outer integument seems to develop prior to the appearance of the inner integument (fig. 28), as stated also by KRATZER (3). As the inner integument begins to differentiate, the primary sporogenous cell can be seen (fig. 1) imbedded in three layers of cells, the two innermost apparently comprising the parietal tissue. The ovule is somewhat curved and the two integuments differentiate when the megaspore mother cell appears imbedded in about five layers of nucellar tissue (fig. 2). At heterotypic division, the haploid number of chromosomes is nine (figs. 3, 4). This agrees with the chromosome count by SUGIURA (6). An axial row of four cells is present, and the chalazal megaspore becomes the functional one (figs. 5, 6). These observations are contrary to the findings of USTERI (10), who claimed that the micropylar megaspore becomes functional, and HEILBORN (1), who stated that no axial row is formed.

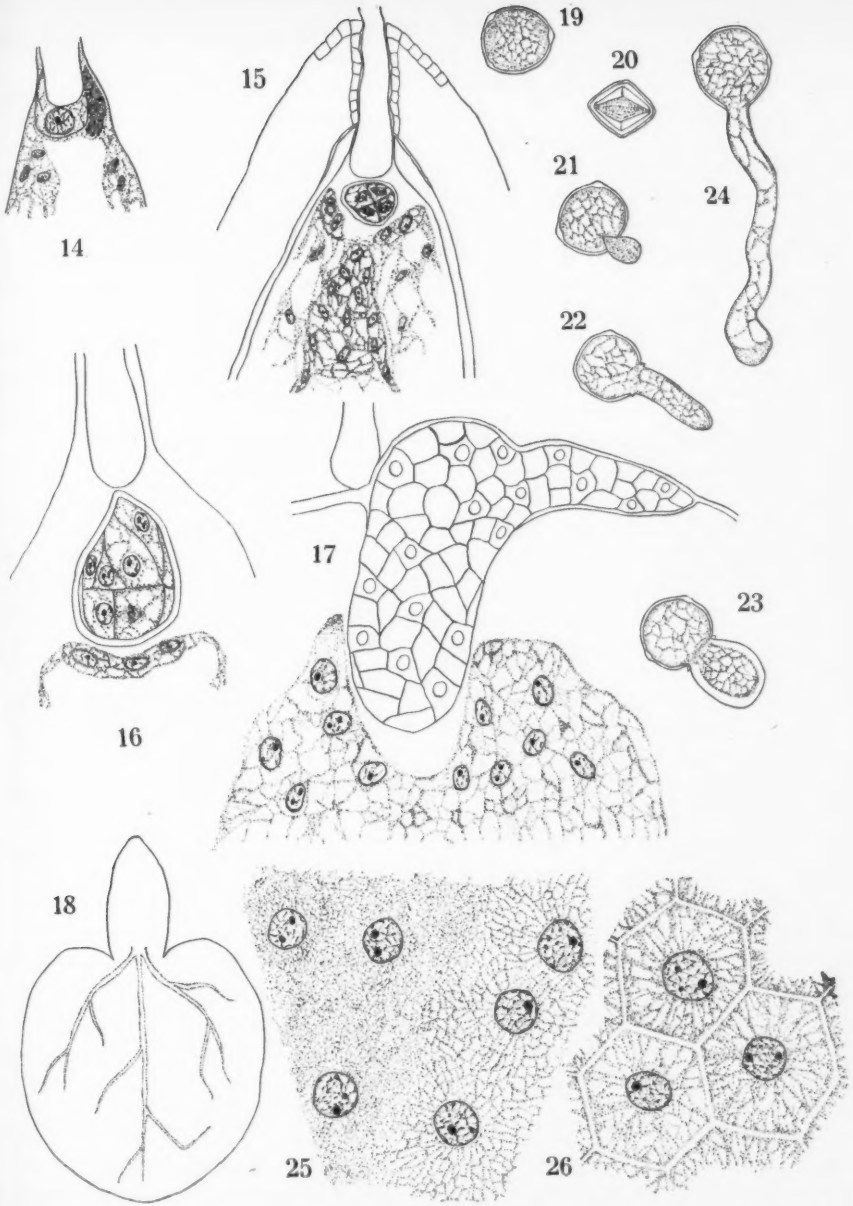
The functional megaspore divides to form a two-nucleate megagametophyte followed by a four-nucleate stage (figs. 7, 8). A seven-celled, eight-nucleate gametophyte of the normal type is formed (fig. 9). This confirms the work of USTERI (10) and KRATZER (3), but is contrary to the findings of HEILBORN (1). The antipodal cells disintegrate almost immediately after their formation and well before pollination occurs. The egg apparatus appears to be extremely minute in comparison with the large embryo sac and massive ovule in which it is located (figs. 10, 30). In the young seven-celled gametophyte, the synergids are about the same size as the egg and are characterized by nuclei at their micropylar ends and conspicuous vacuoles at their free ends. A filiform apparatus develops late in the differentiation of the synergids (fig. 11). The egg cell enlarges considerably at this stage (fig. 12) and contains a large vacuole at the micropylar end. Various stages in megagametophyte development, from two-nucleate to young seven-celled, eight-nucleate forms, are found within different ovules of the same pistil. It is evident that all the thousands of ovules which may be present at a given time within a single pistil are not at the same stage of development.

Under greenhouse conditions the first evidences of pollen tubes within the ovules are found in pistils 10 days after pollination, as compared with the experiments of TRAUB and O'RORK (8), who state that the pollen tubes reach the ovules in the apical end of the pistil within a day and a half and those in the proximal end within 5 days after pollination. The pollen tubes are large massive structures which usually take a rather uniform dark purple stain with Delafield's haematoxylin. A tube grows through the micropyle and becomes slightly arched as it enters the nucellus. The cytoplasmic content seems more granular in some of the tubes as they grow through the micropyle, but no differentiation was observed in the contents of the ends of the older tubes which have reached the nucellus and the gametophyte (fig. 13).

The size of pollen tubes found within the ovules compares favorably with that of the tubes of artificially germinated pollen grains. Germination occurred within one hour after the grains had been placed on an agar-sucrose medium. The tubes, which are unusually large and bounded by relatively heavy membranes, typically become bulbous at the tips (figs. 19–



FIGS. 1–13.—Fig. 1, young nucellus showing primary sporogenous cell and parietal tissue Fig. 2, megaspore mother cell; prophase. Fig. 3, same; heterotypic anaphase. Fig. 4, heterotypic anaphase; enlarged spindle from fig. 3. Fig. 5, interkinesis. Fig. 6, functional chalazal macrospore with three disintegrating megaspores. Fig. 7, two-nucleate megagametophyte; disintegrating megaspores. Fig. 8, four-nucleate megagametophyte. Fig. 9, young seven-celled eight-nucleate megagametophyte. Fig. 10, mature megagametophyte after disappearance of antipodal cells. Fig. 11, mature megagametophyte showing filiform apparatus of synergids. Fig. 12, egg cell of mature megagametophyte from fig. 10. Fig. 13, twisted pollen tube with bulbous tip in contact with young zygote, 16 days after pollination; several endosperm nuclei present.



FIGS. 14–26.—Fig 14, zygote 18 days after pollination showing pollen tube, endosperm nuclei, and disintegrating synergid. Fig. 15, embryo 23 days after pollination showing surrounding sheath; persistent pollen tube and abundant free-nuclear endosperm. Fig. 16, embryo with surrounding sheath 35 days after pollination; persisting pollen tube and free-nuclear endosperm. Fig. 17, many-celled embryo about 64 days after pollination showing sheath, haustorial suspensor, persistent pollen tube, and abundant free-nuclear endosperm. Fig. 18, nearly mature embryo 4 months after pollination showing provascular tissue in cotyledon. Fig. 19, viable pollen grain. Fig. 20, shriveled pollen grain. Figs. 21–24, stages in germination of pollen grains showing bulbous ends of pollen tubes. Fig. 25, beginning of transition from free-nuclear to cellular endosperm, approximately 79 days after pollination. Fig. 26, cellular endosperm.

24). Germination was estimated to be 85–90 per cent.

Fertilization occurred at approximately 13–15 days after pollination. Before fertilization the egg nucleus is quite small, compared with the nuclei of the other cells comprising the mature gametophyte (figs. 10, 11). After fertilization the zygote enlarges considerably and persists for about 5–8 days (figs. 13, 14). There occurs a comparatively great increase in the growth of the fruit during the development of the zygote (table 1). At this stage the end of the conspicuously swollen pollen tube can be seen close to the wall of the zygote and is still traceable back through the nucellus and integumentary region. Some portions of the disintegrating synergids still remain. A few endosperm nuclei are formed, with a comparatively greater number present in the immediate vicinity of the zygote. However, no part of the endosperm is in direct contact with the zygote, which seems to be surrounded by a conspicuous envelope.

There is a definite lag in the development of the embryo, since four-celled embryos do not appear until 23–28 days after pollination (fig. 15). Early divisions occur irregularly and the endosperm grows much more rapidly than the embryo. Ovules approximately 32–35 days after pollination contain embryos with about eight to sixteen cells (fig. 16). Pollen tubes are still persistent at these stages, and all the embryos observed are surrounded by a sheath (figs. 15, 16). The endosperm tissue, still in a free-nuclear condition (although it has increased considerably in amount), is apparently not in direct contact with the embryo.

At about 64 days after pollination there has developed a many-celled, peculiarly shaped embryo which possesses a suspensor-like projection extending off at one side (fig. 17), which appears to be haustorial in nature. The pollen tube still persists at this stage, with its tip closely pressed against the embryo, and it can still be traced back through the integumentary region. The bulbous tip of the

TABLE 1

CORRELATION OF SIZES AND AGES OF FRUITS WITH INTERNAL DEVELOPMENT

Length and width (cm.)	Age	Internal development
	Before pollination:	
1.5 ×0.75	5–7 days	Megaspore mother cell, megasporogenesis, functional megaspore; provascular tissue in funiculus and outer integument of ovule
2.2 ×1.2	2 days	All stages in megagametophyte development; vascular differentiation in funiculus and outer integument
	After pollination:	
2.7 ×1.3	3 days	Mature megagametophyte
3.4 ×1.5	6 days	Mature megagametophyte
3.7 ×1.9	10 days	Pollen tubes entering ovules; well-differentiated vascular tissue in funiculus and outer and inner integuments
3.8 ×1.9	13–15 days	Fertilization
4.0–4.2×2.1 –2.15	16–19 days	Zygote; free-nuclear endosperm
4.5 ×2.4	23–30 days	Four-celled embryo; free-nuclear endosperm; elongated nucellar cells in chalazal region of ovule
4.7–5.0×2.45–2.5	32–35 days	Eight-celled embryo; free-nuclear endosperm
9.5 ×6.3	64 days	Advanced embryo development and abundant free-nuclear endosperm; pollen tube persisting
12.0 ×7.5	79 days	Parts of embryo differentiated; transition from free-nuclear to cellular endosperm
14.2 ×7.9	4 months	Nearly mature embryo; cellular endosperm

pollen tube is similar to those observed by KRATZER (3) and KIRKWOOD (2) in some of the Cucurbitaceae, although structures analogous to the pouches described by these workers were not observed in the pollen tube of *C. papaya*. KIRKWOOD stated that the nutrition of the embryo in some of the Cucurbitaceae was brought about by means of a haustorial pollen tube which obtained its nourishment from the vascular tissue. The abundant endosperm is still free-nuclear, and the embryo appears very minute in comparison with the amount of endosperm present (fig. 33). At this stage the embryo also shows the characteristic film surrounding its upper portion.

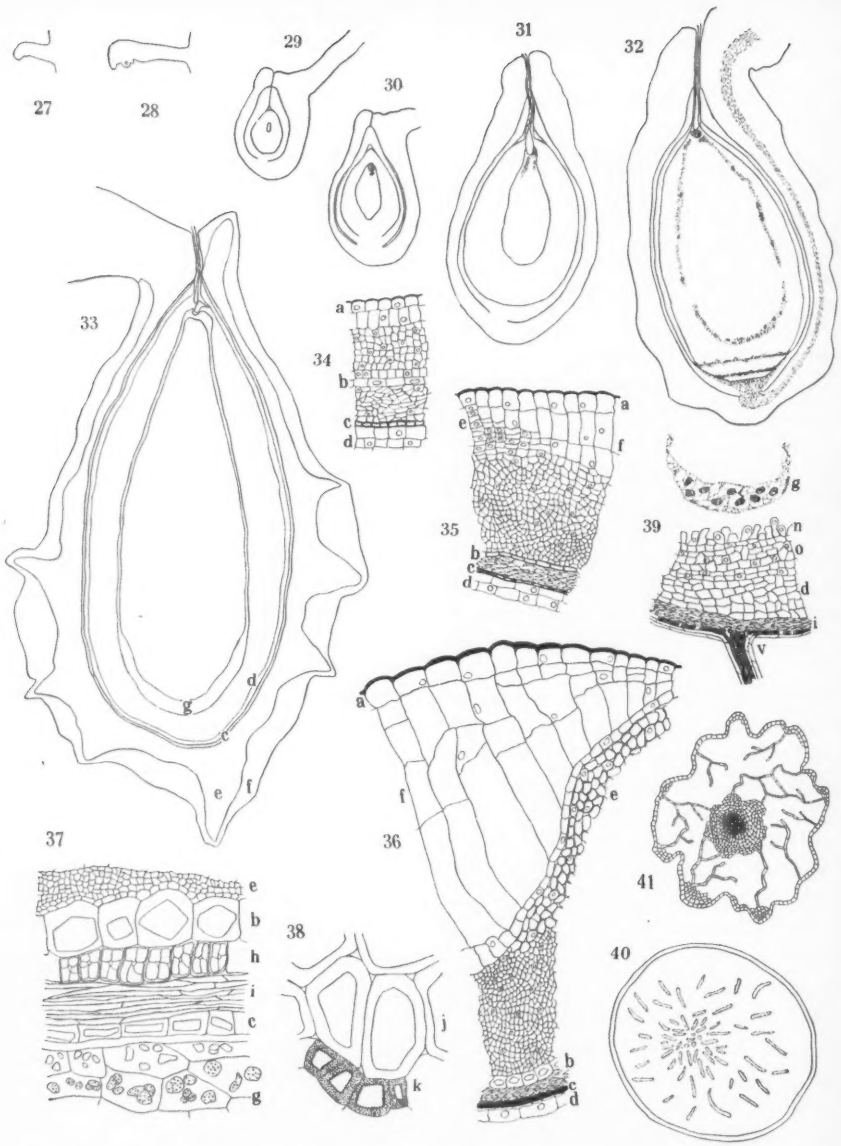
The persistence and position of the pollen tube, the lag in development of the embryo, and the separation of the young embryo from the endosperm, together with the presence of a suspensor, might suggest an unusual nutritive relationship. Although more work is needed to determine the exact interrelationships, it might be suggested that the persistent pollen tube may be closely associated with the nutrition of the embryo in its early stages of development. As the embryo grows older and is more imbedded in the endosperm, there then may occur a shift in the nutritive arrangement and the endosperm might become more functional in relation to the embryo.

Approximately 11–12 weeks after pollination the endosperm shows a transition from the free-nuclear state to a cellular condition (figs. 25, 26). This change occurs toward the micropylar end of the ovule first and proceeds to the chalazal end. The cytoplasm becomes less dense just before cell formation, and numerous minute vacuoles appear within it. After the cell membranes have been laid down, strands of cytoplasm seem to extend from one cell to the next, suggesting division by cell plate formation.

The embryos contained in ovules at 11–12 weeks after pollination have assumed the shape but not the size of the embryo found in the mature seed (fig. 18). The freshly dissected embryos at 4 months after pollination show two broad flattened cotyledons and a comparatively small hypocotyl. The provascular strands in the cotyledons are well differentiated. At this stage the endosperm, which is completely cellular, contains an abundance of aleurone grains and oil (fig. 37), but starch is absent.

Only a very small percentage of the ovules in a given pistil produced embryos in the material studied. This lack of embryo development cannot be due in every case to insufficient pollen, since many ovules which did not develop embryos contain abundant 3-n endosperm tissue, and pollen tubes are also present. In a few instances there is evidence of disintegration of the egg and synergids, even when endosperm nuclei are present. Enzymatic action of the papain present in the ovary wall apparently causes the queer "digested" appearance occasionally seen in the outer tissues of the ovule. Latex tubes are found in abundance in the ovary wall but not in the ovules. In the younger fruits they appear cellular, but in more mature fruits the cross walls apparently disintegrate, leaving long branched tubes. It is very likely that the latex tubes bear some relationship to the vascular system, since they are often located near vascular bundles.

The development of the seed coat was studied in relation to the internal changes observed within the ovules. The seed coat arises chiefly from the tissue of the outer integument, which greatly increases in size in comparison with the inner integument. The inner integument also persists as a distinct layer in the mature seed. During its rapid growth the outer integument becomes differentiated



FIGS. 27–41.—Fig. 27, very young ovule showing nucellus and conspicuous stalk. Fig. 28, section of young ovule at development of primary sporogenous cell; outer integument developing earlier than inner. Fig. 29, anatropous ovule about 2 days before pollination showing massive funiculus, inner and outer integuments, and young megagametophyte. Fig. 30, section of ovule 3 days after pollination containing egg apparatus within large megagametophyte. Fig. 31, ovule 16 days after pollination showing considerable enlargement of outer integument and nucellus; pollen tube, young zygote, and a few endosperm nuclei. Fig 32, ovule 23 days after pollination showing small embryo, persistent pollen tube, abundant free-nuclear

into two distinct parts, the outer layer or sarcotesta and the inner portion or endotesta, as named by STEPHENS (5). The endotesta appears as a compact tissue, which at various intervals forms ridges parallel to the long axis of the seed. The gelatinous transparent sarcotesta, which is composed of large cells, forms the outer portion of the seed coat.

Just before pollination, the two integuments each consist of four or five layers of parenchyma (fig. 29). Three days after pollination, before pollen tubes were observed in the ovules, the outer integument increases slightly in width by means of cell division. There is a slight thickening of the outer walls of the outer epidermis of this integument, and the cells of the inner epidermis of the inner integument show evidence of the deposition of some material, stated by STEPHENS (5) to be tannin (fig. 30).

About 16–19 days after pollination, corresponding to the time of zygote development, all the cells of the inner integument, with the exception of the inner epidermal layer, start to elongate transversely (fig. 34). Further thickening of the walls of the outer epidermal cells of the outer integument is evident (fig. 34*a*), and the outer integument and nucellus are increasing in size through cell division (figs. 31, 34).

When young embryos are present within the ovules, approximately 23–30 days after pollination, the inner integument (except for its inner epidermis) appears as a compact layer between the outer integument and the nucellus. The increase in thickness of the outer integument at this time is due chiefly to the action of meristematic bands, which appear under the outer epidermis and mark the future position of the endotestal ridges (figs. 32, 35*e*). The epidermal and hypodermal cells between the meristematic regions which divide and increase greatly in size (fig. 35*f*) will later become the sarcotesta.

As the embryo attains a later stage of development, about 64 days after pollination, the endotesta and sarcotesta of the outer integument are clearly discernible (fig. 33). The cells of the endotestal ridges have increased greatly in number but are only slightly enlarged (figs. 33,

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endosperm, and vascular strand in outer integument branching into inner integument at chalazal end; irregular endotestal ridges becoming evident in outer integument. Fig. 33, section of ovule 64 days after pollination, still containing comparatively small embryo and persistent pollen tube: *c*, inner integument; *d*, nucellus; *e*, endotesta; *f*, sarcotesta; *g*, abundant free-nuclear endosperm. Fig. 34, section of seed coat 16 days after pollination: *a*, outer epidermis of outer integument; *b*, inner epidermis of outer integument; *c*, inner epidermis of inner integument showing deposition of tannin; *d*, nucellar tissue. Fig. 35, section of seed coat 23 days after pollination. Outer integument: *a*, outer epidermis; *b*, inner epidermis; *e*, meristematic cells later forming endotestal ridges; *f*, large cells resulting from division and enlargement of epidermal and subepidermal cells between meristematic regions, and later becoming sarcotesta. Inner integument: *c*, inner epidermis; *d*, nucellus. Fig. 36, section through seed coat 64 days after pollination. Outer integument: *a*, outer epidermis with greatly thickened outer walls; *b*, inner epidermis; *e*, endotestal ridge; *f*, sarcotesta. Inner integument: *c*, inner cutinized epidermis; *d*, only remaining layer of enlarged nucellar cells. Fig. 37, enlarged portion of mature seed coat. Outer integument: *e*, small thin-walled compact cells; *b*, inner epidermis with greatly enlarged cells and conspicuous crystals. Inner integument: *h*, thick-walled stone cells apparently originating from outer epidermis; *i*, enlarged elongated cells composing great part of inner integument; *c*, inner cutinized epidermis containing tannin; *g*, cellular endosperm containing aleurone grains. Fig. 38, portion of endotestal ridge of mature seed: *j*, greatly enlarged cells; *k*, somewhat smaller cells with pitted walls. Fig. 39, section of nucellus and inner integument at chalazal end of ovule 64 days after pollination: *g*, free-nuclear endosperm; *n*, elongated nucellar cells; *o*, nutritive region of nucellus; *d*, hyaline region of nucellus; *i*, inner integument; *v*, vascular strand. Fig 40, cross section through chalazal region of inner integument 4 months after pollination showing umbrella-like arrangement of vascular elements. Fig. 41, cross section of spongy funiculus 79 days after pollination showing amphicribral vascular bundle.

36). Those cells adjacent to the inner epidermis of the outer integument are small, while those composing each ridge appear progressively larger toward the outermost portion of the ridge (fig. 36*e*). Most of the endotestal ridges lie parallel to the longitudinal axis of the seed. There also occurs a slight increase in the number and a great increase in the size of the epidermal and hypodermal cells located between the ridges (fig. 36*f*), so that the sarcotesta appears transparent and gelatinous in fresh material. Owing to the presence of this layer, the surface of the seed is smooth while it remains within the fruit. The outer walls of the outer epidermal cells become greatly thickened (fig. 36*a*). The inner epidermal cells of the outer integument increase in size, their walls become thickened, and a calcium oxalate crystal is formed in each cell (fig. 36*b*). The inner epidermis of the inner integument is characterized by cells containing an abundance of tannin (fig. 36*c*). All nucellar tissue has apparently disintegrated, with the exception of the outermost layer of enlarged cells adjacent to the inner integument (fig. 36*d*).

In several instances ovules of this age were found, each of which possessed a well-differentiated seed coat without the presence of either embryo or endosperm. Apparently most of the ovules within a pistil develop to a certain extent after pollination, even though fertilization does not occur in some. Changes are seemingly brought about as a result of pollination, which stimulates nucellar and integumentary growth, independent of embryo development.

Although STEPHENS (5) reported the presence of the sarcotesta in the mature seed coat, this region is apparently no longer present in the seed coats of the mature dried seeds studied. The cells composing the tips of the endotestal ridges have been extremely enlarged and possess very thick cell walls (fig. 38*j*), while the innermost region of the endotesta is made up of slightly smaller cells with numerous pits in the walls (fig. 38*k*). The inner epidermal cells of the outer integument still retain their characteristic crystals (fig. 37*b*).

In the inner integument of the mature seed, the outer epidermal layer appears as a row of thick-walled stone cells (fig. 37*h*), and the inner epidermis is composed of greatly enlarged cells containing an abundance of tannin (fig. 37*c*). The other inner integumentary cells are somewhat enlarged and elongated but possess comparatively thin cell walls (fig. 37*i*). These observations concerning the development of the inner integument are contrary to those described by WINTON and WINTON (11), who classify these tissues as an outer layer of longitudinally elongated fibers with thick walls, several rows of transversely elongated fibers, and an inner epidermis of isodiametric cells. The nucellus tissue has completely disappeared, except in the chalazal region.

In tracing the differentiation of the vascular tissue within the ovules, it was found that an ovule at about 6–7 days before pollination contains a single provascular strand which can be traced through the funiculus and the outer integument. However, the ovary wall of a fruit at this age contains well-developed vascular tissue. Some differentiation of the vascular strand into spiral xylem elements and sieve tubes occurs in an ovule approximately 2 days before pollination in the region of the funiculus and outer integument. In more mature ovules, a well-differentiated vascular bundle, amphicribral in type (fig. 41), extends from the funiculus along the endotestal region of the outer integument into the inner integument at the chalazal end, where it

branches widely and irregularly to form an umbrella-like structure (figs. 32, 40).

An interesting specialization of the cells of the nucellar tissue at the chalazal end of ovules is observed 3–12 weeks after pollination. In this region the cells of the nucellus took on the form of rather elongated finger-like processes, unlike the usual nucellus cells (fig. 39*n*). Immediately beneath this modified layer lie two other distinct portions of the nucellus, a dense-staining nutritive region (fig. 39*o*) and a hyaline layer with granular cell contents (fig. 39*d*). Within the inner integument which borders the hyaline region are found the much-branched ends of the vascular strand (fig. 39*v*). Because of the close proximity of the elongated nucellar cells to the endosperm, and also to the vascular strand, some nutritive arrangement is strongly indicated.

Summary

1. In *Carica papaya* the primary sporogenous cell appears within the young ovule as the integuments are beginning to differentiate. One or two layers of parietal tissue are present.

2. The megaspore mother cell is formed simultaneous with the development of the two integuments.

3. Through two meiotic divisions the megaspore mother cell produces an axial row of four megaspores, the chalazal one of which develops into a normal seven-celled, eight-nucleate megagametophyte; the other three megaspores disintegrate. The haploid number of chromosomes is nine.

4. The mature megagametophyte is minute compared with the massive integuments and nucellus. The egg cell enlarges as the megagametophyte grows, and a filiform apparatus develops late in the differentiation of the synergids. The antipodal cells disintegrate almost immediately after their formation.

5. Under greenhouse conditions pollen tubes enter the ovules about 10 days after pollination. Fertilization occurs approximately 13–15 days after pollination. Zygotes persist for about 5–8 days before they divide. Divisions occur irregularly in the young embryos. There is a definite lag in the embryo development of this species, while endosperm development is very rapid, as is differentiation of the seed coat.

6. The large massive pollen tube persists within the embryo sac up to at least 64 days after pollination. The fact that it is closely pressed against the many-celled embryo may suggest a nutritive relationship between it and the young developing embryo.

7. At approximately 79 days after pollination the endosperm shows a transition from the free-nuclear state to a cellular condition, beginning at the micropylar end of the sac. Mature endosperm contains an abundance of oil and aleurone grains, but starch is absent.

8. The seed coat is derived chiefly from the outer integument of the ovule, which grows considerably to form the firm compact endotesta and a transparent gelatinous sarcotesta.

9. The vascular bundle in a more mature ovule extends from the funiculus along the outer integument into the inner integument at the chalazal end of the ovule, where it branches widely to form an umbrella-like structure.

10. Modified elongated nucellar cells at the chalazal end of the ovule, located relatively close to the endosperm and also to the vascular tissue, may play an essential role in some nutritive relationship existing between the entering food supply and the endosperm.

11. Only a very small percentage of the ovules in a given pistil produce embryos, even though endosperm and pollen tubes are present. However, an ovule may grow and develop to a certain extent after pollination, even though fertilization does not occur.

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CURRENT LITERATURE

The Boletaceae of North Carolina. By WILLIAM CHAMBERS COKER and ALMA HOLLAND BEERS. Chapel Hill: University of North Carolina Press, 1943. Pp. viii+93. Illustrated. $7.00.

Despite their relatively large size and conspicuous coloring, the boletes are among the more difficult of the fungi to determine satisfactorily. Aside from spore characters, many of the criteria by which the various species are distinguished are ephemeral and must be observed in fresh specimens. The present volume, with its numerous excellent illustrations, many of them colored, will be of service far beyond the limits of North Carolina, since the great majority of the species discussed are of wide distribution.

The treatment is extremely conservative. All boletes are referred to the three long-established genera—*Boletus*, *Boletinus*, and *Strobilomyces*. No reference is made to recent revisions of the family other than occasional citation of MURRILL's and SNELL's names as synonyms, and such citation is far from complete.

There are two keys. In the first, emphasis is placed on features likely to be noted by the experienced collector, with color subordinated; in the second, color dominates. Some species will doubtless lend themselves better to one than to the other; together they should prove more helpful than either alone.

Three new species and four new varieties, all in *Boletus*, are proposed without Latin diagnoses; hence they are technically *nomina nuda*. In addition, there are four new combinations, three in *Boletus* and one in *Boletinus*.—G. W. MARTIN.

The Design of Experiments. By R. A. FISHER. London: Oliver & Boyd, 1942. Pp. xii+236. 12/6 net.

The third edition of this excellent work is only slightly enlarged from the second edition, published in 1937. The two sections which have been added are those numbered 45.1 and 45.2. These deal with more complicated experimental designs and bear the titles: General systems of confounding in powers of 2; and Double confounding. These sections will enable investigators to plan more satisfactory experiments where many factors are involved. The consistent use of this book should enable agricultural workers, particularly, to improve the design of field experiments, in which numerous factors have to be considered. The emphasis upon design is good, especially for those beginning experimental work.—C. A. SHULL.

ERRATA

Professor T. C. CHIN has just informed us that, owing to a misreading of the stage micrometers, some of his data in the Vol. **104**, June, 1943, issue, "Cytology of the autotetraploid rye," were incorrectly given and should be changed as follows:

P.627, Table 1:

For "No. of stomata per 0.01188 sq. mm." *read* "No. of stomata per 1.846 sq. mm."

For "2.56" *read* "49.35"; *for* "3.14 × 2.18" *read* "44.85 × 62.61" (first line of table).

For "3.26" *read* "55.84"; *for* "3.72 × 2.98" *read* "52.54 × 68.73" (second line of table).